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Seabird Oil Toxicity Study

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FINAL REPORT

SEABIRD OIL TOXICITY STUDY

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ABSTRACT

This study investigated short-and long-term effects of oil exposure on three species of seabirds: Cassin's Auklet (Ptychoramphus aleuticus), Common Murre (Uris aalge), and Wedge-tailed shearwater (Puffinus pacificus).

Laboratory investigations evaluated the acute toxicity of oil to Cassin's Auklets and Common Murres. Auklets were exposed to 3-5 ml of weathered Santa Barbara crude oil, and murres were obtained from the wild after becoming oiled in spills at sea. Oiled alcids preened and ingested oil. Weathered Santa Barbara crude and Bunker C oils both caused toxic reactions, including liver cell disassociation, hemosiderosis, renal tubular necrosis, and Heinz body hemolytic anemia, but did not result in marked intestinal irritation, adrenal hypertrophy or salt gland hypertrophy. Scanning electron microscopy detected Heinz bodies in erythrocytes 3-4 days after exposure to Bunker C oil. Hematocrits averaged 22% (range 14-32%) compared to a normal hematocrit of 55-60%.

Oil selected for field studies was Santa Barbara crude, Ames #3120-9, a Monterey formation sour crude. Oil was weathered artificially for 4-6 days to develop products similar to weathering under ocean spill conditions. Weathered oil did not penetrate the plumage of birds or cause loss of waterproofing.

Field studies with auklets were carried out on Southeast Farallon Island (SEFI), 40 km west of San Francisco, using 500 artificial nesting burrows. Shearwater studies were conducted on Manana I., 1.2km offshore from Oahu, HI. Several hundred natural burrows were marked in a 60 X 80 m plot.

Cassin's Auklets were dosed with 1 ml weathered oil either orally by gelatin capsule or externally by application to the breast plumage. Auklets were dosed prior to lay or during incubation. All birds were dosed only once, and breeding success and mate fidelity were observed for 2-4 years. A high proportion of auklets dosed externally with oil prior to egg laying abandoned breeding. Those birds remaining delayed egg laying by more than 20 days. The delay appeared to be a consequence of disruption of egg formation and a delay in initiation of growth of new ovarian follicles. Oral dosing was less severe than external dosing. Auklets exposed externally to oil on day 14 or 15 of incubation had a high frequency of abandonment, low hatching success, and low net breeding success. Eggs which became oiled during incubation had lower hatching success than comparable controls.

Oil exposure caused greater abandonment of auklet pairs breeding for the first time than for established pairs, and resulted in fewer female auklets returning in the year following exposure, but did not change the proportion of males returning. Breeding failure resulted in many birds changing mates in the second year. Mate switching resulted in lowered success in hatching and fledging the first egg, reduced re-laying attempts and lowered success in the year after dosing. Pairs which remained together in the year after dosing had a higher re-laying frequency after the failure of their first egg.

Shearwaters were dosed orally with 2 ml or externally with 2.0, 1.0, 0.5 or 0.1 ml of weathered crude oil applied externally to the breast plumage of both members of a pair. Oil dosing was timed to precede the pre-laying

exodus. Oil exposure resulted in most birds abandoning the colony. Length of the pre-laying exodus was not changed in birds which did return. Oil exposure at all levels caused lowered egg laying and hatching success, with complete breeding failure at 2.0 ml. None of the eggs from birds externally dosed with 2 ml hatched. Birds dosed orally (2.0 ml) were affected less, but chicks of orally dosed birds had significantly poorer survival than control chicks.

The net breeding success of both oral (2 ml) and external (2 ml) groups was lower than that of the control group in the second year, suggesting long-term breeding depression after exposure to a single small dose of oil. Fewer externally oiled birds returned, fewer laid eggs, and net production of chicks was lower than for the other treatment groups. Birds returning with their original mates laid eggs with higher frequency, a greater percentage of those eggs hatched, and a higher percentage of chicks fledged compared to birds breeding with a new mate in the year after dosing. This was true for all groups taken together and for each separate treatment group.

The data strongly support the hypothesis that breeding failure after oil exposure resulted in mate switching which caused overall lowered breeding success in both Cassin's Auklets and Wedge-tailed shearwaters.

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EXECUTIVE SUMMARY

This study investigated both short- and long-term effects of single small exposures of weathered Santa Barbara crude oil on two species representing different taxonomic groups of seabirds: Cassins' Auklet (Ptychoramphus aleuticus), a northern lattitude alcid specialized for diving; and Wedge-tailed shearwater (Puffinus pacificus), a tropical procellariiform specialized for long flights in search surface prey which are seized on the wing or captured in shallow plunge-dives. Wedge-tailed shearwaters are not a species common in California waters, but they are closely related to Sooty shearwaters which are extremely common migrants and summer residents along the California coast. Acute toxicity studies of both crude and refined oils were conducted with a second alcid species, the Common Murre (Uris aalge). Most of the effort was directed to field studies in which doses of oil were fed to adult, breeding birds, or doses were applied to their feathers. The birds' subsequent reproductive performance was determined over a 2-4 year period. Supporting laboratory investigations evaluated the acute toxicity of oil including clinical symptoms and histopathological examination of exposed tissues.

Studies with auklets were carried out on South East Farallon Island (SEFI), 40 km west of San Francisco, California. Five hundred artificial nesting burrows were installed on SEFI because access to large number of natural burrows was difficult. shearwater studies were conducted on Manana Island 1.2 km offshore from Oahu, Hawaii, in a colony of more than 20,000 birds nesting in burrows dug into volcanic tuff soil. Several hundred natural burrows were marked in a 60x80 m plot.

The oil selected for study was Santa Barbara crude oil Ames well #3120-9, Holly platform, a Monterey formation, "sour crude", supplied by ARCO Inc. to Marine Biological Consultants, Costa Mesa, CA. Two oil samples were used from pump dates in March, 1981 and October, 1982. Samples of fresh and weathered oil were analyzed by Science Applications Inc., La Jolla, CA. Weathered oil was desired which did not penetrate the plumage of the birds and cause loss of waterproofing.

Oil was weathered under controlled conditions to produce products similar to weathering uncle r ocean spill conditions. Experimental weathering uncle r controlled conditions was carried out at University of California, Davis (UCD). Small batches of fresh crude oil were weathered by laying 100 ml onto the surface of 100 L of 3.5% NaCl in tap water. The water was stirred constantly and maintained at outside ambient temperature of 6-15°C. Oil was shaded from direct sun during weathering to prevent photodecomposition. We chose to weather the oil with as little alteration in chemical composition as possible and arrive at a weathered product which differed from the fresh crude by loss of volatile and water soluble components, but without the creation of additional, unknown polynuclear aromatics and polymers. The selection of a particular viscosity of weathered oil was based upon wetting and buoyancy experiments carried out with domestic ducks. Weathered oil for field studies was prepared in two 1000 ml batches and weathered to the same viscosity as a trial batch of oil which did not penetrate the plumage of exposed ducks.

Cassin's Auklets and Common Murres were studied under captive conditions to assess acute toxicity of ingested oils. Auklets captured on SEFI were exposed to 3-5 ml of weathered oil for a broad survey of the overall effects of oil on alcids. Murres were obtained from the wild after becoming oiled in a Bunker C

spill. Oiled auklets preened and ingested oil and some intestinal segments were dilated and contained black material which smelled of oil. Damage to the livers of auklets and murres consisted of disassociation of hepatocytes and accumulation of Prussian Blue positive iron pigment in hepatocytes and Kupffer cells. Acute renal tubular necrosis was found only in birds exposed to oil. Random renal tubules, particularly distal collecting tubules, contained epithelial cells with granular or vacuolated cytoplasm and pyknotic nuclei.

The preliminary study provided histopathological information on wild alcids to small quantities of weathered Santa Barbara crude or Bunker C oil. Although these oils were of quite different composition, they caused similar toxic reactions, including liver cell disassociation, hemosiderosis, renal tubular necrosis, and Heinz body hemolytic anemia, but did not result in marked intestinal irritation, adrenal hypertrophy or salt gland hypertrophy.

Additional studies were conducted with Common Murres at rehabilitation centers after they had been exposed to a variety of types of spilled petroleum in the wild. These studies concentrated on anemia caused by ingestion of petroleum while preening oil from their fouled plumage. Scanning electron microscopy was used to detect the presence of Heinz bodies (Hemoglobin aggregates) present in the red blood cells of murres 3 to 4 days after the birds were exposed to Bunker C oil and cleaned. Hematocrits of these 8 oiled murres ranged from 14-32% with an average of 22%. Normal hematocrit of murres is 55-60% of blood volume. The anemia represented a loss of 40-75% of the circulating red blood cells. Blood was also obtained from 5 control and 7 dosed auklets as part of the 1984 field study. A blood smear from each bird was examined by scanning electron microscope. One of three externally dosed birds and three of four orally dosed birds had mild red blood cell changes consisting of aggregates of hemoglobin beneath the red cell surface and small protrusions similar to the pinching off of membrane blebs for extrusion of Heinz bodies.

Cassin's Auklets occupying nest boxes on SEFI were dosed with 1 ml weathered oil either orally by gelatin capsule or externally by application to the breast plumage in 1982 and 1984. Auklets were dosed prior to lay in two experiments in 1982 and 1984 and were dosed during incubation in a third study in 1984. All birds were dosed only once and the breeding success and mate fidelity were observed for 2-4 years. The year 1983 was a severe El Nino year with complete breeding failure of all monitored nest boxes and no auklets were dosed with oil in that year.

A high proportion of auklets dosed externally with oil prior to egg laying responded by abandoning the breeding season. The remaining birds were delayed in egg laying by more than 20 days in both the 1982 and 1984 studies. The delay appeared to be a consequence of disruption of egg formation and a probable delay in the initiation of growth of new ovarian follicles. Oral dosing results were less severe than external dosing, with only a short delay in egg laying and no effect on laying frequency, hatching success, or fledging success. Auklets exposed externally to oil on day 14 or 15 of incubation exhibited a high frequency of abandonment, low hatching success and low net breeding success. Eggs which became oiled during incubation had lower hatching success than comparable controls, indicating the possibility of direct embryo toxicity of oil transferred to eggs by incubating auklets.

Oil exposure caused greater abandonment of pairs breeding for the first time in nest boxes than for established pairs. Exposure to oil during incubation caused breeding failure, but many established pairs remained together and

laid a second egg. The net breeding success of this group was higher than for new pairs, both as a result of less abandonment of the first egg and because of a much higher relaying frequency. Pairs which remained together in the year after dosing demonstrated the same trend, with a higher relaying frequency after the failure of their first egg.

Oil exposure resulted in a lower proportion of female auklets returning in the year following exposure, but no change in the proportion of males returning. Breeding failure resulted in many birds changing mates in the second year. Changing mates resulted in lowered success in hatching and fledging the first egg, reduced relaying attempts, and lowered success in the year after dosing.

Shearwaters responded to oil exposure with results similar to the auklet Study. In 1983, 61 pairs were dosed orally with 2 ml weathered oil in gelatin capsules, 60 pairs were oiled externally by spreading 2 ml weathered oil evenly over the central breast plumage, and 115 pairs of controls were sham-dosed. An additional 192 pairs of unhanded birds laid eggs in marked burrows during the 1983 study and were used as a secondary control group. A second experiment was conducted with shearwaters in 1984, but using 1.0, 0.5 or 0.1 ml of weathered crude oil applied externally to the breast plumage of both members of a pair. Thirty pairs of previously unclosed shearwaters were dosed at each level of oil. Oil dosing in both years was timed to precede the shearwaters' pre-laying exodus. The time of dosing allowed us to observe: (1) the effects of oil on delay or interruption of egg formation; (2) the effect of stress on strength of pair bonds; and, (3) the nest site tenacity of returning birds.

Oil exposure did not change the length of the pre-laying exodus of the birds that did not return, but resulted in a majority of oiled birds abandoning the colony. Oil contamination of shearwaters resulted in significant lowering of egg laying and hatching success after only 0.1 ml weathered Santa Barbara crude oil was applied to the breast plumage. Stepwise increases in exposure yielded progressively reduced breeding, with complete breeding failure occurring at an amount of 2.0 ml. Birds dosed orally with 2.0 ml oil in gelatin capsules were also adversely affected but to a lesser extent.

It is not known whether oil dosing had a direct effect on egg formation as demonstrated with Cassin's Auklets. It is unlikely that there was an effect, however, as no delay in egg laying was observed. None of the eggs of birds externally dosed with 2 ml hatched and chicks and orally dosed birds had unexplained but significantly poorer survival than control chicks. It is possible that some of the reduced survival of embryos and chicks resulted from toxicity of oil residues incorporated into eggs during yolk formation.

Externally (2 ml) oiled birds displayed reduced breeding success in the year after treatment, suggesting that long-term breeding depression may result when seabirds are externally exposed to a single small amount of oil. Significantly fewer oiled birds returned and incubated eggs in the study plot in the year after exposure. The net breeding success of both oral (2 ml) and external (2 ml) groups was lower than that of the control group. Birds externally oiled with 2 ml were most affected by the long-term effects of a single exposure to oil. A smaller percentage of externally oiled birds returned, a lower percentage laid eggs, and the net production of chicks was lower than for the other treatment groups.

Birds returning with their original mates laid eggs with higher frequency, a greater percentage of these eggs hatched, and a higher percentage of chicks

fledged compared to birds breeding with a new mate in the year after dosing. This was true for all groups taken together and for each separate treatment group. The summary data for all groups is consistent and shows that 80% of all birds not laying eggs in 1983 changed mates in 1984, while 85% of all birds successfully raising a chick remained together.

The data strongly support the hypothesis that breeding failure of dosed birds as a result of oil application resulted in mate switching, and that mate switching resulted in overall lowered breeding success.

The most striking effects of oil observed in our field studies were the combination of abandonment and decreased mate fidelity of both auklets and shearwaters. Birds which abandoned before egg laying usually failed to return to breed in subsequent years or returned with different mates. Birds which remained throughout the breeding season after being dosed had reduced breeding success and most returned with a different mate in the following year, Changing mates resulted in a trend toward reduced breeding success in the year after oil exposure.

Auklets and shearwaters are long-lived seabirds with prolonged breeding season, and a coordinated effort on the part of both adults is necessary for successful reproduction. A substantial amount of learning is probably associated with breeding success, as older birds are usually more successful, and maintenance of the pair bond improves breeding success in most species of seabirds. Disruption of this pair bond by oil exposure results in lowered breeding success for more than one year.

I. INTRODUCTION

A. Background and Objectives

Increasing attention to offshore oil drilling, leasing Outer Continental Shelf (OCS) submerged lands, oil tanker traffic, and the probability of accidental oil spills has produced a need for understanding impacts of such activities on human, marine, and coastal environments. Various legislative acts have directed the Minerals Management Service (MMS) to develop balanced managerial plans for the development of OCS resources. There is also a need for an adequate data base to support efforts to prepare environmental impact statements under the requirements of the National Environment Policy Act (NEPA). Some of these requirements and the authority to address them have been delineated in the California OCS Environmental Studies Plans prepared by the Pacific OCS office of the MMS. The MMS has initiated a number of studies as part of such plans. This project is a component of a series of studies dealing with the OCS resource development activities.

The impacts of spilled oil on seabirds have always been difficult to assess. After a spill in which birds are heavily oiled, the total mortality is greater than the number of carcasses counted, perhaps 5 to 10 times greater (Clark 1973, Page et al. 1982). The bulk of the birds sink, or are carried away by currents and wind. The hidden nature of the effects of oil may be similarly underestimated when birds contact sublethal amounts of oil. Alternatively, such sublethal effects may be transient, allowing birds to recover with almost no long-term effects. Seabirds are generally long-lived, with low reproduction rates. Their breeding strategies have evolved to cope with occasional disruptions of their breeding due to environmental conditions. However, chronic reductions in fecundity or repeated breeding failure has profound effects on the population, and recovery is extremely slow.

B. Study Purpose

The objective, as specified by the MMS, has been: "to determine the long-term sublethal effects of three concentrations of ingested crude oil on seabird reproduction for two selected sea bird species in the field". Accordingly, the study investigated toxic effects of ingested weathered Santa Barbara crude oil on Cassin's Auklets (Ptychoramphus aleuticus) and Wedge-tailed shearwaters (Puffinus pacificus), with emphasis on long-term changes in reproductive success. Most of the effort has been directed to field studies in which oil doses were fed to adult breeding birds or were applied to their feathers. The birds' subsequent reproductive performance was determined over a 2-4 year period. Supporting laboratory investigations evaluated the acute toxicity of oil including clinical symptoms and histopathological examination of exposed tissues.

The overall approach required a number of sequential activities to realize the final objective. Specifically:

- Two species of sea birds with different foraging habits and risks of exposure to oil were selected from many species of sea birds which breed or migrate along the California Coast. Species ultimately selected were Cassin's Auklet and Wedge-tailed shearwater. Although Wedge-tailed Shearwaters neither migrate nor breed in California waters, this representative species of shearwater was selected based upon access to a breeding colony in U.S. waters.

- Field site selection was based on feasibility of conducting long-term research. The sites were: Southeast Farallon Island, CA. (SEFI) for Cassin's Auklets, and Manana Island, HI. for Wedge-tailed shearwaters.

- Crude oil from offshore Santa Barbara, CA, Ames well 3120-9, Monterey foundation, was selected for the study.

- Weathered crude oil was selected for field studies for two reasons: (a) to realistically simulate the probability that most birds would encounter oil with some weathering after a spill; and, (b) to minimize the acute risk of penetration and wetting of the plumage of exposed birds.

- Oil was weathered under controlled conditions to produce products similar to weathering under ocean spill conditions. Weathered oils were evaluated in preliminary experiments and a six-day weathered product was selected which did not penetrate the bird's plumage and cause loss of waterproofing.

- Preliminary experiments were conducted on captive Cassin's Auklets to determine the exposure levels of oil to which birds could be subjected.

- Artificial nest-boxes for Cassin's Auklets were constructed and placed on SEFI to facilitate field observation of a large sample of dosed and control birds.

- Auklets breeding in artificial nest-boxes, and shearwaters breeding in natural burrows, were banded for future identification. The breeding success of control and dosed birds was determined during the first year of oil exposure and over one to three subsequent breeding seasons.

II. STUDY PLAN

A. Species and Field Sites Selected for Study

Nineteen seabird species breed along the California coast (Sowls et al. 1980), and many are vulnerable to spilled oil, especially alcids. In addition, many other species spend their non-breeding periods in California waters. Some occur in immense numbers and of the latter, shearwaters are also vulnerable to spilled oil. Some which nest only in concentrated colonies at one or two locations are most likely to be damaged by a spill from an accident at a drilling platform, from a tanker or cargo ship, or from a pipeline. It would be desirable to know how oil affects the reproduction of such birds as albatrosses, storm-petrels, shearwaters, murres, puffins, auklets, murrelets, cormorants, and pelicans, but the number of species potentially available for experimentation is severely limited. Most species nest in dense colonies on exposed surfaces of small islands or on cliffs which prevent close approach to birds for banding, dosing, repeated identification, and monitoring the growth rates of chicks. Burrow-nesting seabirds are more accessible and a dense colony can be censured repeatedly without disturbing the entire colony. For these reasons, only the species nesting in burrows were considered. We determined from preliminary field studies that Cassin's Auklet (Ptychoramphus aleuticus) and Wedge-tailed shearwater (Puffinus pacificus) were nest-suited for long-term study.

At the onset of this project, background information needed for a satisfactory experimental design was available for only one species, Cassin's Auklet. The breeding biology of Auklets had been studied, including its responses to acute effects of oil ingestion in regard to timing of egg formation, egg production, matchability, fledging success, and other aspects of reproduction (Ainley et al. 1978, 1981; Grau et al. 1977). Extensive experience with this species permitted the design of field studies likely to yield accurate and useful data. When the study began, there was no other seabird, free-living waterfowl, or shorebird for which this was true.

The selection of a suitable second species was difficult because it was desirable to study a species not closely related to auklets. Cormorants and pelicans nest in California but have the serious drawback of abandoning their nests when approached. This severely restricts the ability to band birds, and precludes repeated capture, dosing, and making subsequent observations. Gulls were a possible choice, but previous attempts to study effects of oil on them were unsuccessful (Ainley et al. 1979). Preference was therefore given to a procellariiform species.

Shearwaters or other Procellariiformes are rarely encountered as beached birds after an oil spill even though flocks of several hundred thousand may be offshore from a particular spill. That shearwaters may avoid oil slicks has been suggested by Varoujean et al. (1982). Some procellarids, however, actually ingest oil from the surface of the ocean, however, which indicates a lack of avoidance (Boersma 1986). An alternate possibility to explain the few recoveries of shearwaters may be their strictly pelagic habits. Birds encountering oil far out at sea may not be able to swim to the shore, and some strictly aquatic species, such as Eared Grebes, refuse to swim ashore even when oiled. Such a response by hundreds of Eared Grebes was noted on several occasions during the cleanup and rescue operations following the spill of the Tanker "Puerto Rican", both at South

East Farallon Island (SEFI) and in Bodega Harbor. Several Northern Flumars (Fulmarus glacialis) (3 live, 16 dead) were recovered from beaches after the "Puerto Rican" spill. Aerial surveys of birds at risk from the slick identified only small numbers of fulmars and no shearwaters within 3000 m of shore (Page and Berkner, 1985). The fulmar carcasses were heavily waterlogged indicating they were probably oiled further to sea than other beached birds. Whether the number of oiled fulmars was representative of the population at risk, whether fulmars drowned and sank, or whe the r most fulmars were able to avoid oil is unknown. Shearwaters, similarly, may be reluctant to swim to beach themselves.

Although shearwaters and storm petrels are among the most common birds in California waters, only storm petrels breed in California; their colonies, however, are too small to support a study such as ours. Leach's Storm petrels (Oceanodroma leucorhoa) which breed in large colonies in Oregon were selected as a possible second species.

Shearwaters do not breed in California. The Sooty shearwater (Puffinus griseus), which is the most common seabird in California waters during the spring, summer, and fall, breeds in Chile, Australia, and New Zealand. The closely related Wedge-tailed shearwater (Puffinus pacificus) breeds in easily accessible, large colonies in the Hawaiian Chain. This is the only species of shearwater which breeds in large colonies on U. S. territory; hence, this species was selected as an alternative species to Leach's Storm petrels, even though Wedge-tailed shearwaters are rarely observed in California waters.

B. Field Sites Selected for Study

1. Cassin's Auklet

Previous studies with auklets had been carried out on SEFI, 40 km west of San Francisco, where there is a breeding population exceeding 100,000 birds. Several areas of the island appeared suitable for the installation of 500 artificial nesting burrows (see Figure II-l). The artificial burrows constructed for auklets were of dimensions similar to natural burrows: an entrance of 3 x 3 1/2 inches, overall length of 18" with an offset nest chamber 8 x 8 x 6 1/2 inches at the rear of the 10" entrance tunnel (Figure II-2). Nest boxes were used on SEFI because much of the terrain is very rocky and access to large numbers of natural burrows on steep terrain is difficult.

The facilities on SEFI were excellent, although access to the island is often difficult in the winter and spring because of heavy seas and the absence of a pier. A boom and cargo net is used to off load personnel and supplies. Point Reyes Bird Observatory (PRBO) maintains a field station on the island year round, and resident biologists Study birds and marine mammals. PRBO collaborated in this study by coordinating field studies and providing biologists for daily monitoring of auklet breeding and for collecting samples.

2. Leach's Storm petrel

Two field sites were attempted in 1982. Saddle Rock, Oregon, is a high-density colony with shore access and was possibly suitable for the installation of artificial nesting burrows. Access to the islet, however, proved impractical because of a climb up the side of the rock and the necessity of camping overnight in an exposed area. Wading to shore was possible only at low tide and climbing the rock at night was unsafe.

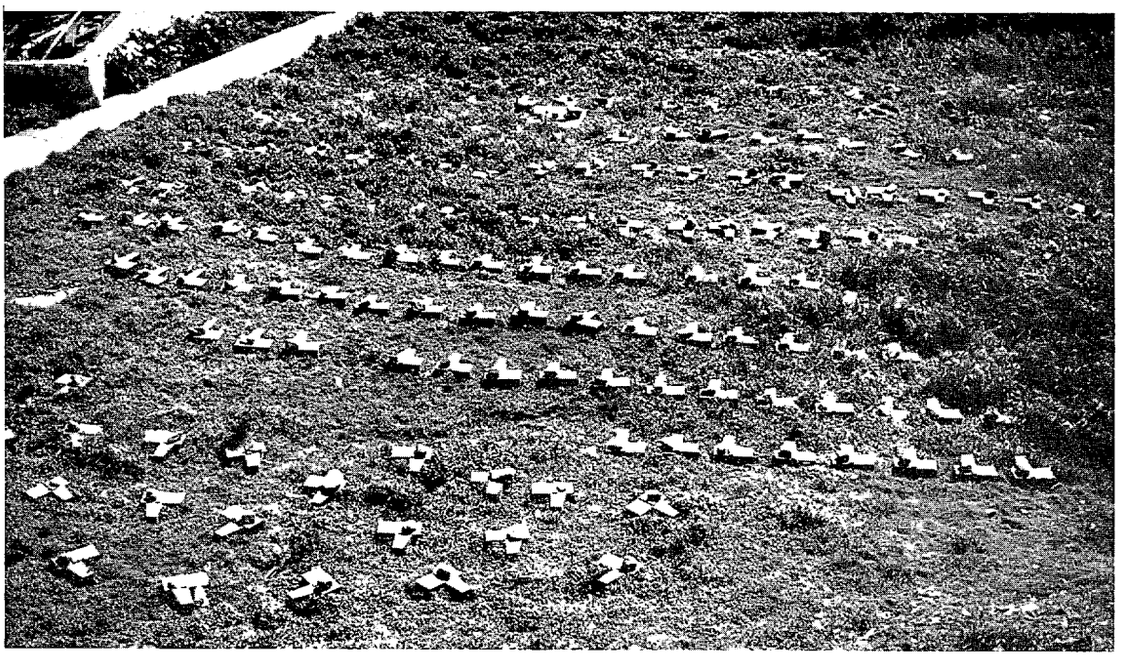
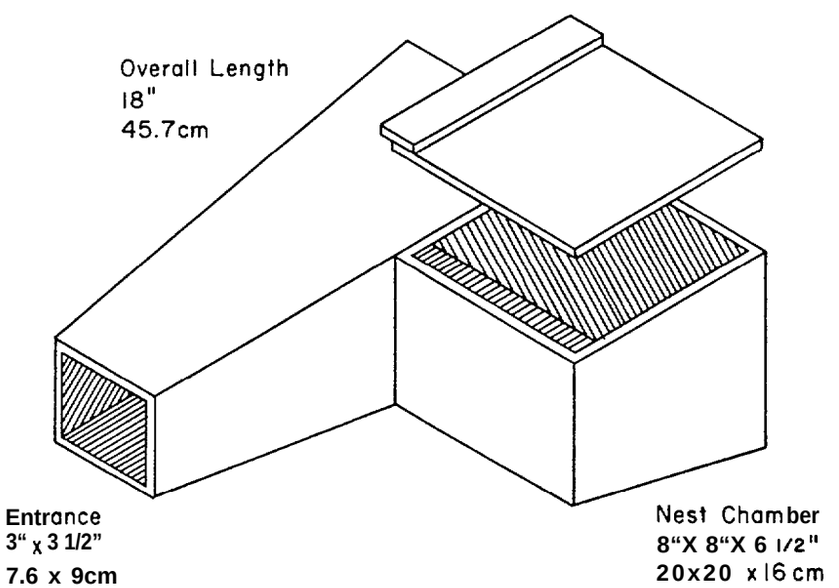


Figure II-1. Artificial nest boxes on South East Farallon Island, CA.



CASSIN'S AUKLET NEST BOX

Figure II-2. Cassin's Auklet nest box.

The second offshore island selected was Hunter Island, Oregon, six miles south of Gold Beach (Figure II-3). Access to Hunter Island required an inflatable boat and was hampered by poor landing conditions on both the mainland and island. An experienced field biologist of the Oregon Institute of Marine Biology, was retained to manage the preliminary work. With his assistance, 200 artificial nest boxes designed for storm-petrels were installed and monitored on Hunters Island.

The 200 nest boxes were constructed based on a successful pattern used by PRBO for Ashy Storm-petrels used on SEFI. The boxes had an entrance of 1 1/2 x 1 3/4 inches, a length of 24" and were tapered so that the nest chamber at the rear of the box was 3" high and 5" wide (Figure II-4). The boxes had slightly larger entrances and were longer than Ashy Storm petrel boxes to allow for the larger body size of Leach's and their habit of excavating deeper burrows.

Nest boxes were transported to Oregon in April, but installation was delayed until May as the choice of field site was changed after exploration of Saddle Rock and Hunters Island.

Access to Hunters Island was by an inflatable boat launched from a boat ramp six miles north at Gold Beach, because shore conditions and surf prevented launching from shore adjacent to the island. The 200 nest boxes were installed on Hunters Island along the periphery of the breeding colony to prevent destruction of many natural burrows.

Hunters Island was visited on June 8, 1982 to check occupancy and insure that boxes were intact and correctly placed. Some of the 197 boxes were repositioned to seat them properly in the soil. Three boxes were not found, due to the dense, high grass. Only one box was occupied on June 8. Boxes were again checked on June 19, but only one pair of birds was incubating an egg. Some other boxes showed evidence of use (feathers in the boxes), but no additional eggs were laid during the 1982 season. The single pair abandoned the box after the June 19th visit.

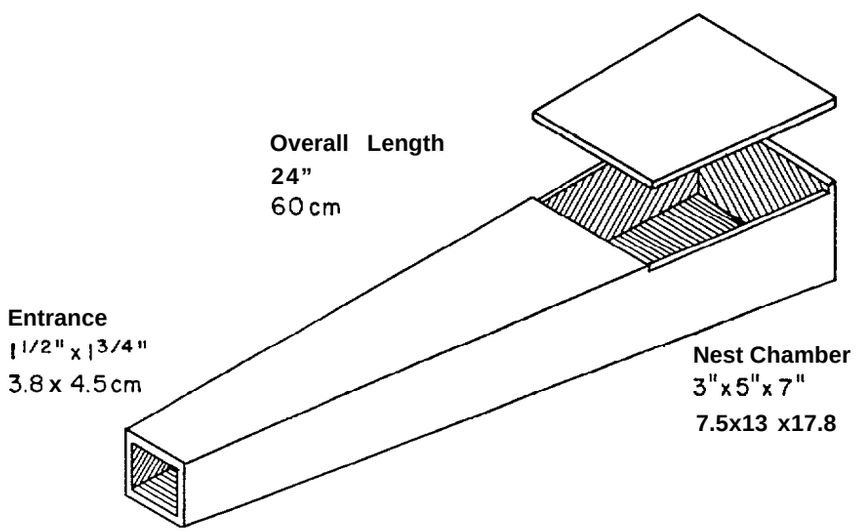
Low occupancy of boxes by Storm petrels was expected in the first year, since we expected at least one breeding season for birds to become accustomed to the boxes. As many as 50% of the natural burrows on Hunters Island were unoccupied on June 20, indicating a large excess of natural nesting sites, hence there was no pressure on birds to move into artificial boxes. The low occupancy of boxes, and desertion of the single pair of incubating petrels, indicated that this species was unlikely to be a successful second species for this Study .

3. Wedge-tailed Shearwater

Wedge-tailed shearwaters were the second procellariid species selected for preliminary studies in 1982. Wedge-tailed shearwaters are not a species common in California waters. But, they are closely related to sooty shearwaters which are extremely common migrants and summer residents along the California coast.

Wedge-tailed Shearwaters breed in colonies throughout the tropical Pacific. One particularly favorable site from the standpoint of access and nearby facilities is Manana Island (Rabbit Island), a small

Figure 11-3. Hunter Island, OR.



LEACHS STORM-PETREL NEST BOX

Figure II-4. Leach’s Storm-petrel nest box.

volcanic islet 1.2 km offshore of Oahu, Hawaii (Figure II-5). The island is near the Oceanic Institute where facilities were contracted for housing and laboratory space. Makai Research Pier adjacent to the Institute provided excellent facilities for storage and mooring of an inflatable boat. Manana I. is a Hawaii State Bird Refuge with access limited to organized research teams and has been the site of many field studies of both shearwaters and Sooty Terns. More than 20,000 shearwaters nest in burrows on the island and several hundred natural burrows suitable for using for field research could be marked in a 60 m by 80 m plot (Figure 11-6).

c. Oil Selected For Study

The choice of crude oil was most important, as it had to be representative of oil pumped from California OCS platforms. The oil selected was Santa Barbara crude, Ames well #3120-9, Holly platform, a Monterey formation, "sour crude". It was supplied by ARCO to Marine Biological Consultants (MBC) in Costa Mesa, CA. Two samples of one and five gallons each were supplied to us by MBC, representing pump dates in March 1981 and October 1982. Samples were characterized by MBC and a chemical analysis of the fresh crude was performed by Science Applications Inc, La Jolla, CA. Results of the analysis are presented in Section IV-B.

1. Weathering of Crude Oil

The oil composition and physical properties, as well as method of administration of oil to birds, were principal concerns during the initial phases of this Study. An oral dose is an artificial situation, as may be the application of fresh crude to the plumage of a bird. However, since the oil spilled on the sea becomes quickly weathered, changing its composition and physical properties, and because the majority of birds are unlikely to be oiled within a few hours after a spill, studies of the effects of weathered crude oil was judged to be more appropriate than those employing fresh crude.

Oil spilled on the ocean surface rapidly forms a thin slick with a large surface area and begins to lose volatile components to the air and polar components into the water. Wave action and mixing result in a weathered product of increased viscosity which, through time, eventually coalesces as tar balls. Experimental weathering under controlled conditions was carried out at University of California, Davis (UCD). Small batches of fresh crude were weathered by laying 100 ml onto the surface of 100 liters of 3.5% NaCl in tap water. The water was stirred constantly and maintained at outside ambient temperature of 6-15°C during December 1981 and January 1982. The initial oil layer of approximately 0.5 mm thickness coalesced as weathering progressed. Batches were weathered for 1, 3, 5, and 6 days, with the resulting viscosity increasing markedly. The weathering process was temperature dependent. Batches weathered at warmer temperatures increased in viscosity more rapidly than in cold weather; the viscosity of the weathered product could also be adjusted by altering weathering time.

All weathered products contained much trapped water which separated very slowly from the oil. Centrifugation at 1000 x g separated the oil and water effectively but with considerable loss of oil due to the adherence of weathered product to the centrifuge bottles.

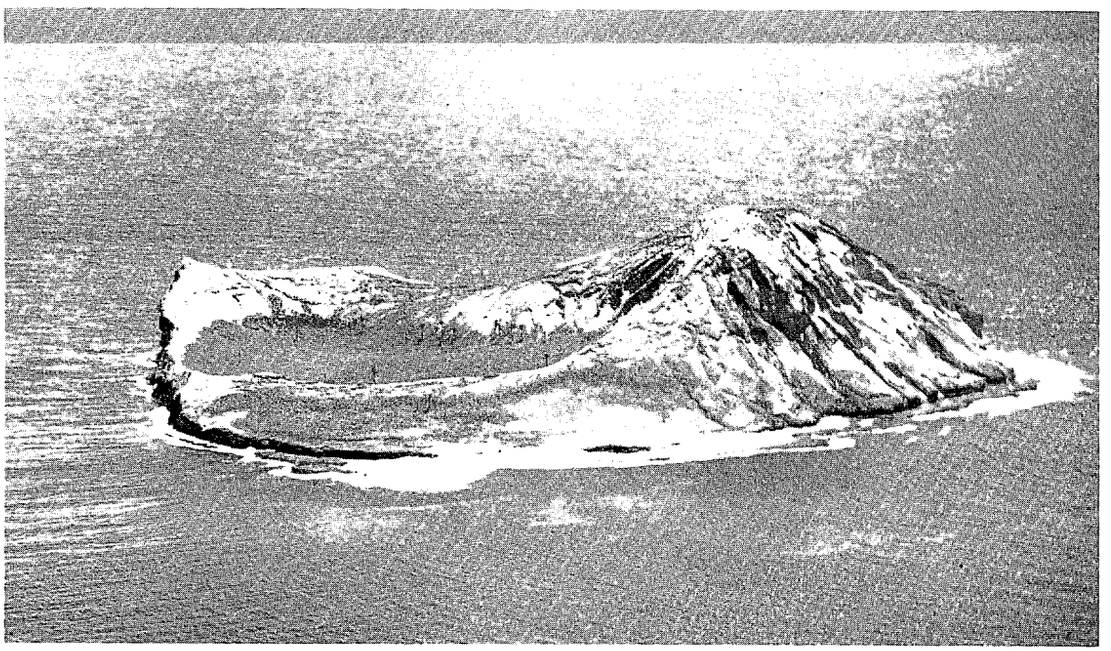


Figure II-5. Manana Island, Oahu, HI.

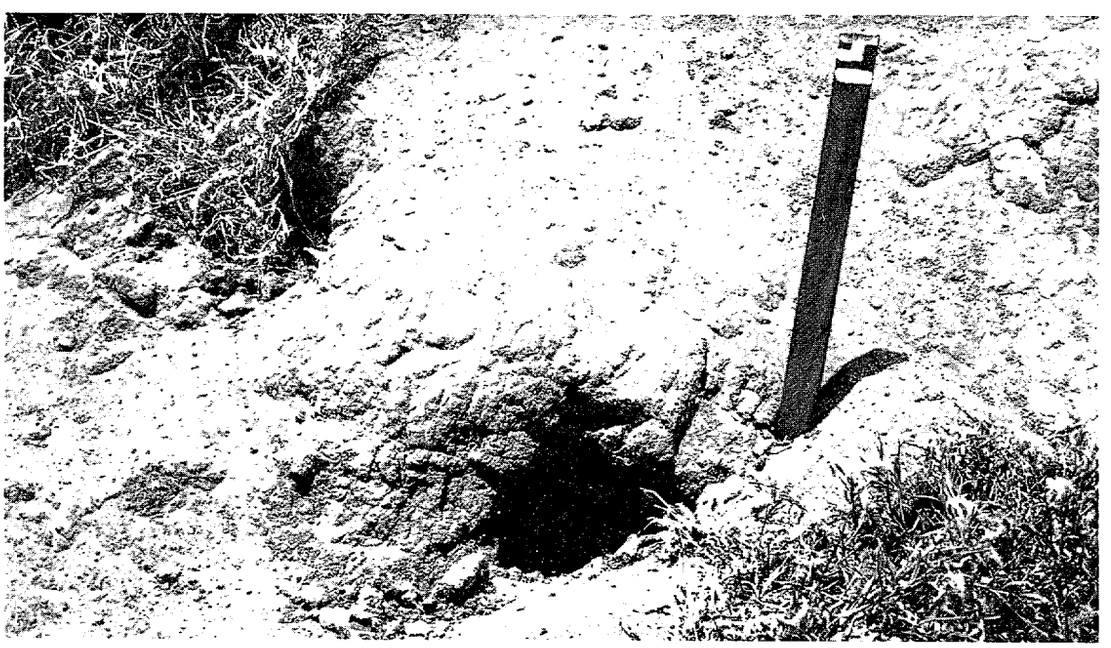


Figure II-6. Marked shearwater burrow on Manana Island.

The selection of a specific viscosity of weathered oil was based upon wetting and bouyancy experiments carried out with domestic ducks as discussed in Section V-A. After selection of a suitable product for use in field experiments, two large batches of weathered oil were prepared by layering 1 liter samples of oil on the surface of 1,000 liters of artificial sea water and weathering for 4 and 5 days outside in the shade. Shading from direct sun was done to prevent photodecomposition of the oil. The intent was to weather the oil with as little alteration in chemical composition as possible and arrive at a weathered product which differed from the fresh crude by loss of volatile and water soluble components, but without the creation of additional, unknown polynuclear aromatics and polymers. It is likely that the weathered oil thus produced could be less toxic than oil weathered in the sun in which a larger proportion of more toxic aromatics would be created through photooxidation.

The final batch of crude was weathered to a viscosity similar to that of the six-day weathered product found to be the least penetrating to the plumage of ducks used in the buoyancy experiments described later in Section V-A.

Samples of a high-viscosity four-day weathered crude were analyzed by gas chromatography and mass spectrometry by Science Applications Inc. This batch of weathered oil was used for the field studies of 1982. The analysis and procedure are given in Section IV-B.

III. ACUTE TOXICITY OF OILS TO AUKLETS AND MURRES

A series of studies were conducted with Cassin's Auklets and Common Murres under captive conditions to assess acute toxicity of ingested oils. One study was conducted with auklets captured on Southeast Farallone Island (SEFI) and exposed to controlled quantities of weathered Santa Barbara crude oil. Four additional studies were conducted with Common Murres at rehabilitation centers after they had been exposed to a variety of types of spilled petroleum in the wild. The initial study was a broad survey of the overall effects of oil on alcids, the later studies concentrated on the severe anemia caused by ingestion while preening oil from their fouled plumage.

Three separate oil spills occurred off central California during this study. A small Bunker C spill of unknown origin occurred in June 1983, when the tanker "Puerto Rican" spilled 1.5 million gallons 11 miles south of SEFI on November 3, 1984, and when the barge "Apex Houston" spilled approximately 20,000 gallons of San Joaquin crude oil in February 1985. Each of these spills was important from the standpoint of possible interference in the field studies. The type of oil and acute toxic effects on oiled birds were investigated to compare with the results of acute studies on auklets to document possible effects on auklets in the field. The "Puerto Rican" spill was particularly important as spilled oil came ashore on the Farallones and could have been disastrous to auklets.

A. Studies of Acute Toxicity to Captive Cassin's Auklets and Common Murres

Cassin's Auklets were brought into captivity to determine the acute toxic effects of oil resulting from ingestion of oil preened from their feathers. This controlled experiment provided an assessment of toxicity with lowered risk of death from thermal stress. The captive study also provided needed background information of wild birds for histopathological examination to document the extent of natural injuries and parasitism which could contribute to reduced survival following oil exposure.

In addition to controlled Cassin's Auklets experiments, seven Common Murres exposed in an accidental Bunker C oil spill were examined for comparison with the acute toxic effects of Santa Barbara weathered crude and the extent of natural injury and parasitism to be encountered in the wild murre population. The murres had been exposed in an offshore oil spill of Bunker C and were collected April 4, 1982, cleaned by Bird Rescue Research Center, Berkeley, CA., and transported to Davis for this study. Hematocrits were determined upon arrival at University of California, Davis (UCD), and the birds were euthanized immediately (10 days after initial oil exposure).

Six adult auklets were captured on SEFI on January 22, 1982, sexed by bill measurement, weighed, banded with color bands and transported off the island by boat and taken to UCD. Four additional auklets sacrificed on the island as controls and tissues, were fixed in buffered formalin for histological examination. One of the six auklets taken off the island died in transit to UCD. Tissues from that auklet were also fixed for histological examination and the carcass frozen. The remaining 5 birds were released into a room provided with artificial nest boxes for temporary shelter and a 2,000 l salt water pool. The birds were initially observed for 1 hour to insure they were healthy before the lights were turned off. The room was kept at 12°C, lights on at 7:00 a.m., off at 6:00 p.m. to simulate conditions on SEFI during January.

Auklets were provided with previously frozen, thawed euphasiid shrimp, live fresh water minnows; and previously frozen, thawed night smelt ad libitum. No birds ate during the morning of January 23 and force feeding was begun January 23. Average weight loss of the auklets was 18 gm (11% of average body weight) during the first 28 hours after capture (Figure III-1). The auklets were observed continuously during the light hours through a small window from an adjacent room. Birds were force-fed 4 times per day, weighed once per day, and cloacal temperatures taken once per day. All birds lost weight throughout the preliminary experiment.

On January 24, the birds appeared stressed. The room temperature was raised to 19.5*C and a heat lamp was installed in one corner of the room to allow the birds to warm themselves as they chose. Increasing the temperature resulted in more activity and improved appearance of the birds.

The birds were divided into 2 groups on January 25 for oil application. Two birds were kept as controls, three were oiled. Five ml of weathered crude oil was applied to the breast feathers of one bird (BLUE) on January 25. Dosing of the other two experimental (RED and GREEN) was delayed for 24 hours to observe the extent of oil transfer from BLUE to the other birds. Both RED and GREEN were extensively oiled within 1 hour of BLUE being oiled because the birds huddled together. The control birds (YELLOW and ORANGE) were separated from the oiled birds with nylon netting to prevent them from becoming oiled. Small blood samples were taken from YELLOW, ORANGE, and BLUE on January 25 to check hematocrits (Yellow = 60%, Orange = 66%, Blue = 60% of blood volume), RED and GREEN were each oiled with 3 ml on January 26. RED was oiled on the dorsal surface of both wings and GREEN was oiled on the breast plumage. The primary and secondary feathers of RED became completely matted and non-functional within hours. Application of oil to the wings of a bird in the wild would result in a wet, matted, flightless bird which would almost certainly die of exposure or predation. Three ml of oil applied to the breast plumage of GREEN resulted in a thick coating of the entire ventral surface of the bird. The weathered oil did not penetrate the plumage, however, and GREEN and BLUE both remained buoyant when swimming.

RED and GREEN both died January 28 between 9 and 11 a.m. YELLOW, ORANGE and BLUE were taken alive to the Necropsy Laboratory of the Veterinary Medicine Teaching Hospital. Blood was drawn for hormone analysis and clinical chemistry. A post-mortem examination of all 5 auklets was performed by Dr. Linda Lowenstine, Dept. of Veterinary pathology, at which time tissue samples were taken for histological examination (Figures III-2 and III-3).

1. Hematology

   Hematocrits from 5 auklets taken 14 hours after capture ranged from 60-66% of blood volume with a mean of 62%. At the time of euthanasia, 7 days after capture, the hematocrits of the two control auklets were 36% and 48%, and 21% for the one oiled auklet. Hematocrits of 6 of the murres, which were bled prior to euthanasia (14 days post oil exposure), ranged from 43-52%. The published normals for California Common Murres are 50% (Lefant et al. 1969) and 55.8% for Atlantic Common Murres (Bradley and Threlfall, 1974).

2. Post-mortem Analysis of Auklets

   The complete gross necropsy and histopathology reports are included as Appendix A.

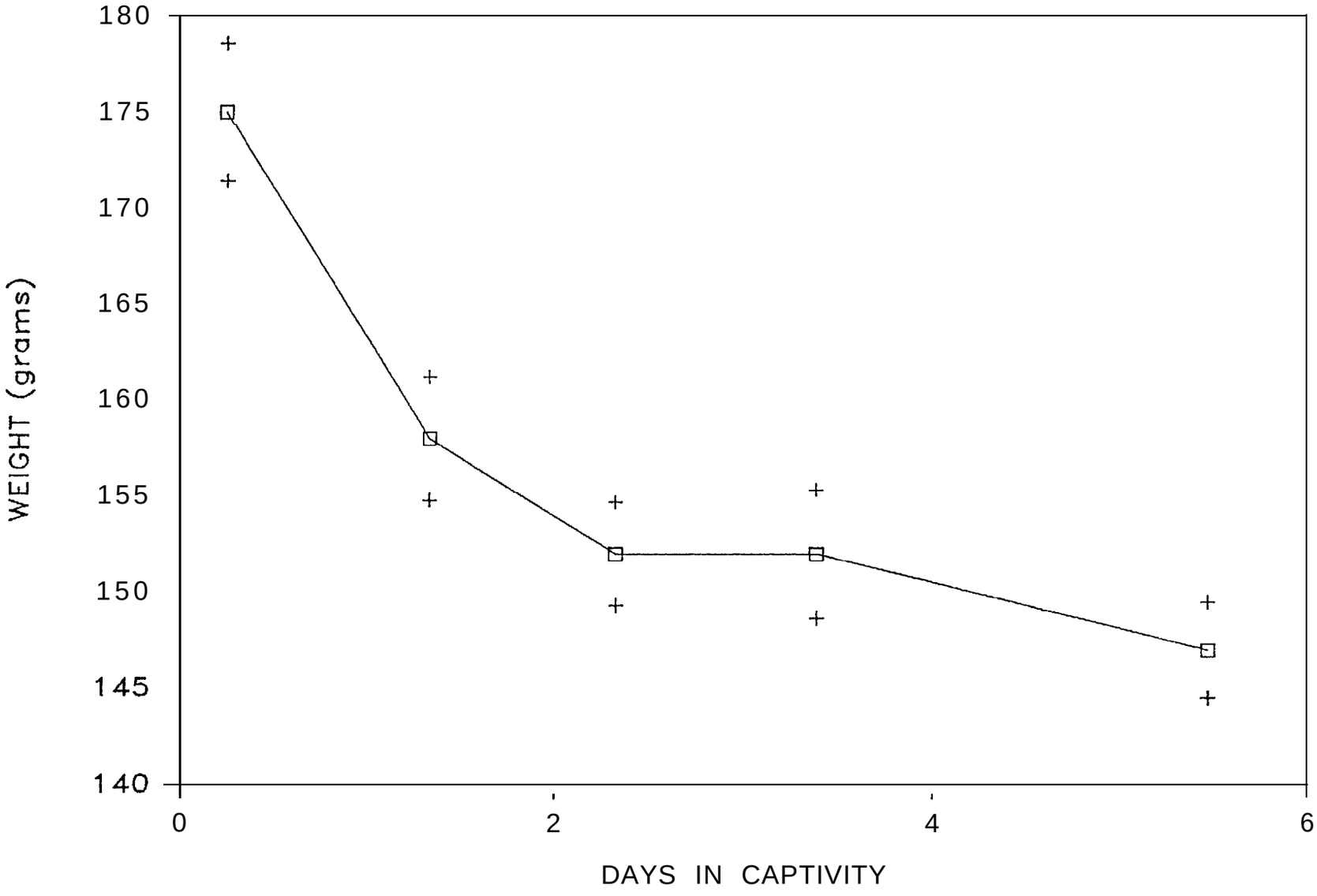


Figure III-1. Average body weights of captive Cassin's Auklets.

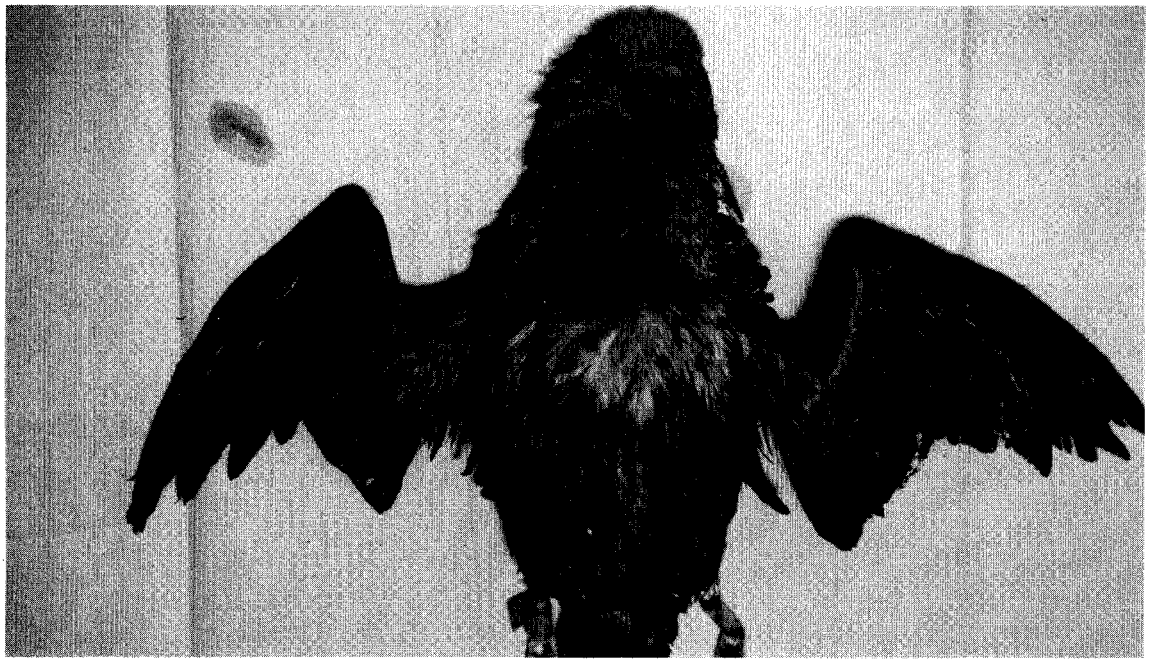


Figure III-2. Auklet RED at necropsy.

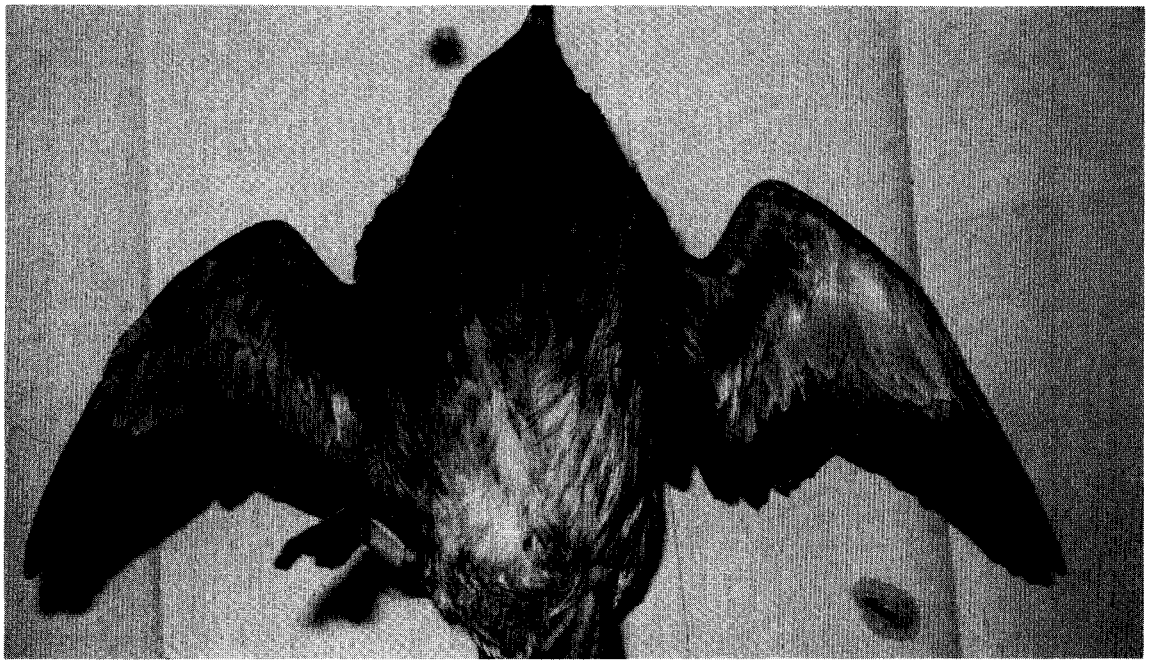


Figure III-3. Auklet BLUE at necropsy.

Lesions were seen in various tissues from all birds examined whether oiled or control (Table III-l). At gross necropsy observation, all birds had multiple small oval to linear ulcers and erosions and opaque yellowish-white plaques in the upper gastrointestinal tract. Histologically, these were foci of necrosis in esophagus, crop, proventriculus, and ventriculus (Figures III-4, III-5), sometimes associated with granulomas and heterophilic inflammation extending deep into the submucosa and focal areas of the superficial cells of the crop and esophageal mucosa which were swollen. Foreign body or parasitic trauma was the suspected cause. In one auklet, a fluke was present on the proventricular mucosa, but otherwise no etiologic agent was observed.

Intestinal segments of oiled auklets were dilated and contained black material which smelled of oil. These contents were negative for occult blood. Histologic sections of the intestines revealed pigmented lumenal contents and mild degeneration of villus tips indistinguishable from autolysis.

Damage to the livers of auklets consisted of disassociation of hepatocytes and by the accumulation of Prussian blue positive iron pigment in hepatocytes and Kupffer cells (Figures III-6 to III-9) These changes were not seen in the four control auklets sacrificed on SEFI, but were seen in two control birds kept in captivity for seven days. Mild hemosiderosis without disassociation was present in the one bird which died en route between SEFI and U.C. Davic.

Acute renal tubular necrosis was found only in those auklets exposed to oil. Random tubules contained epithelial cells with granular or vacuolated cytoplasm and pyknotic nuclei (Figure 111-10). These changes were most marked in distal collecting tubules in the medullary cones.

Salt glands in all auklets were of expected size and were morphologically normal. The ratio of adrenal "cortical" to "medullary" cords was slightly greater than 1:1 in all auklets examined (N = 6) except the one bird which died in transit. In this bird, the ratio was more nearly 1:1, suggesting that mild cortical cell hypertrophy was present in all auklets held in captivity whether exposed to oil or not. No increase in adrenal size was observed microscopically.

An additional histologic finding in lungs of 9 of the 10 auklets was the presence of nodules of macrophages containing golden brown to black partially birefringent crystalline material within intraatrial septa of tertiary bronchi and adjacent to secondary bronchi (Figure 111-11). In some cases, these aggregations were accompanied by lymphoid hyperplasia and focal fibrosis. The pneumoconiosis was more marked in males, perhaps reflecting a greater role in digging and maintaining breeding burrows. Lung flukes (probably Typhlocoelom sp.) were present in one of the oiled auklets.

3. Post-mortem Examination of Murres

Examination of the seven oiled murres revealed some of the changes observed in the oiled auklets. One notable exception was that the murres, which are cliff (not burrow) nesters, had no dust particles in their lungs. Lung flukes were seen in one murre.

Table III-1: Summary of histopathological findings from control and oiled alcids. Cassin's Auklets were oiled experimentally with weathered Santa Barbara crude and Common Murres were oiled in an accidental spill of bunker C.

TISSUES EXAMINED	NUMBER OF BIRDS WITH ABNORMALATIES (Number affected/Number examined)		
	control auklets[a]	oiled auklets	oiled murres
A. Liver: hepatocellular dissociation	2/7	3/3	0/7
hepatocellular iron	2/7	3/3	6/7
Kupffer's cell iron (hemosiderosis)	3/7	3/3	5/7
B. Kidney: Tubular necrosis	0/7	3/3	3/7
C. Lung: Pneumoconiosis	6/7	3/3	0/7
D. Parasitism:	1/3[b]	1/3	5/7
lung flukes	0/3	1/3	1/7
gastric flukes	1/3	0/3	0/7
nematodes (peritoneal, gastric)	0/3	0/3	3/7
coccidia (proximal intestine)	0	0	4/7
cestodes	0	0	1
unidentified ova (urinary epitheliums)	0	0	2/7
E. Multifocal necrosis of crop, esophagus, proventriculus, or ventriculus	4/6	3/3	5/7
F. Other infectious problems			
1. Viral inculsion bodies (kidney, salt gland)	0 / 7	0/3	2/7
2. hepatitis	1/9	1/3	1/7
3. pancreatitis	0/6	0/3	2/7

[a] One control auklet died in transit to UCD from the Farallones; 2 were kept in captivity at UCDfor 7days; 4 were killed in the wild.

[b] Complete tissues not examined from four auklets.

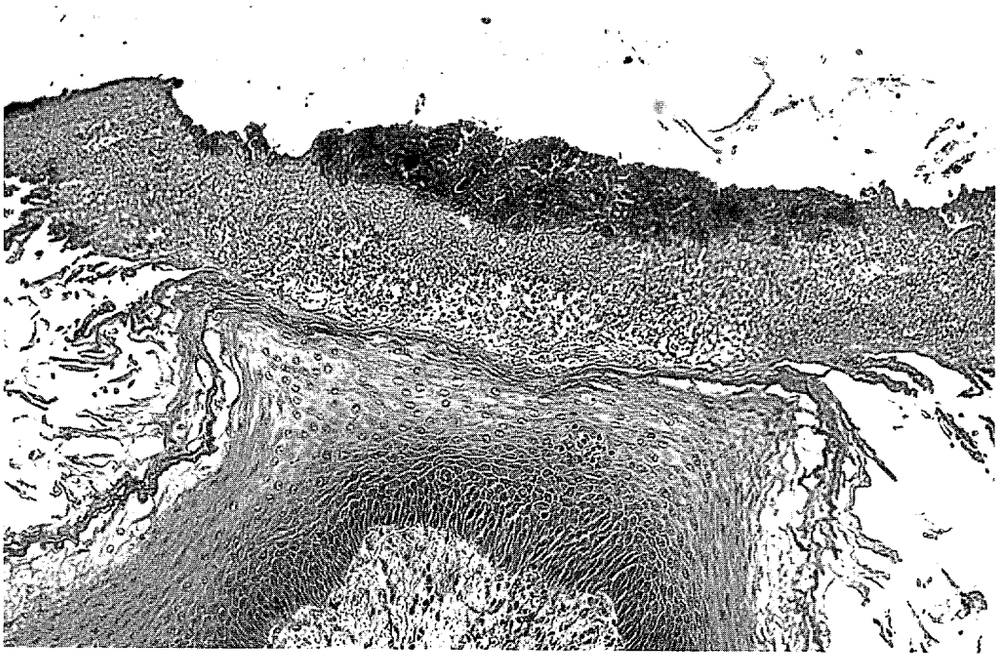


Figure III-4. Necrotic plaque in upper gastrointestina 1 tract (crop) of an auklet. Such plaques were common in both oil-exposed and control birds. Hematoxylin-eosin (H and E), 150X.

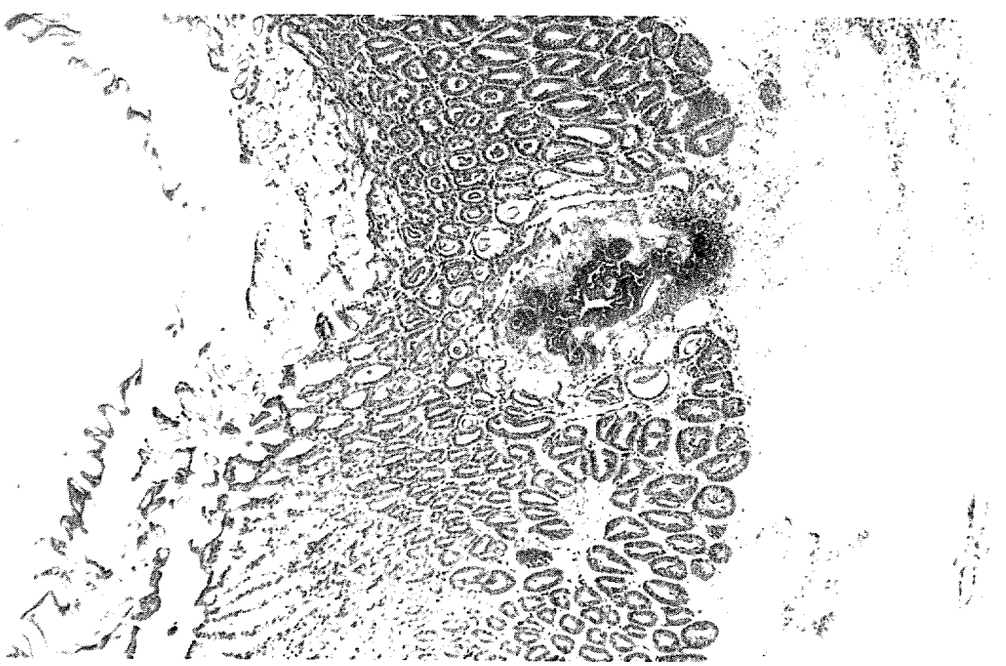


Figure III-5. Focus of deep necrosis in ventriculus of control auklet associated with moderate inflammation. H and E, 80x.

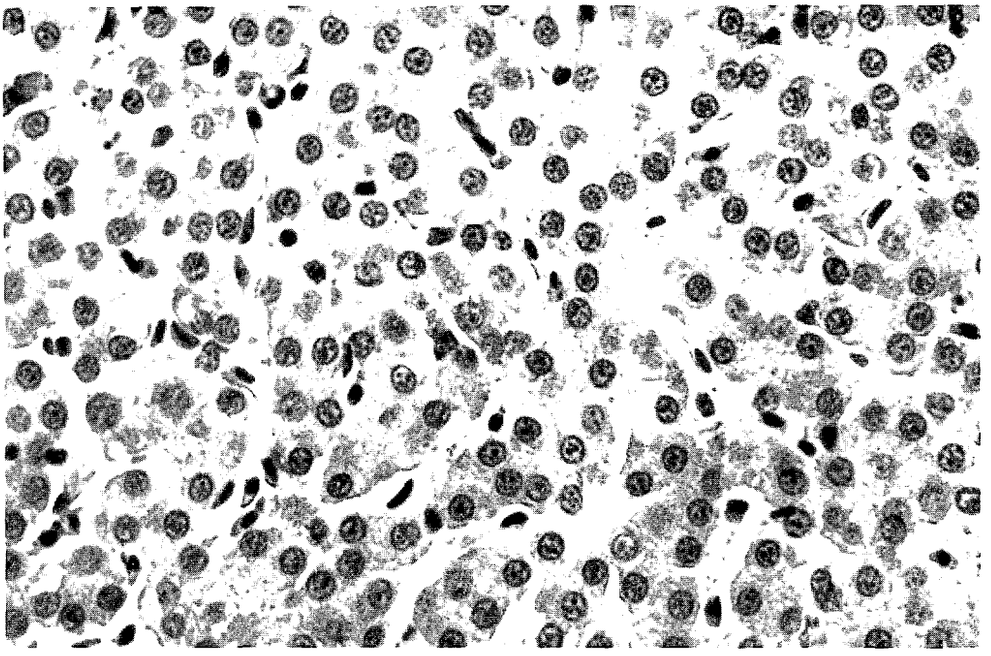


Figure III-6. Liver from a control Cassin's Auk let. Hepatocytes are arranged in cords separated by sinuses containing nucleated red blood cells. H and E, 630x.

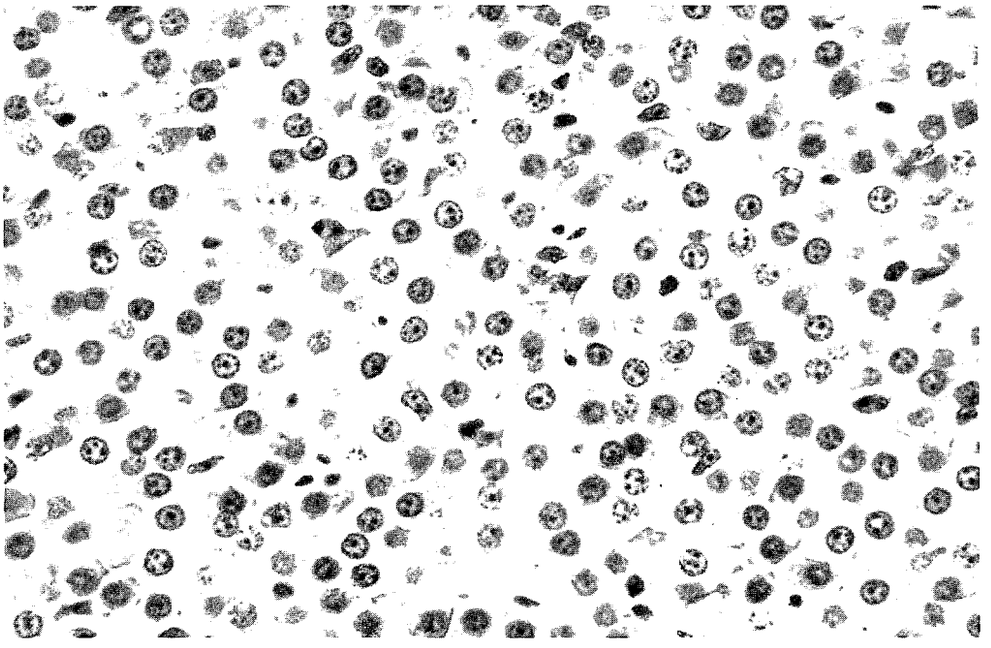


Figure III–7. Disassociation of hepatic cords with individualization of hepatocytes in an auk let exposed to oil. H and E, 630x.

Figure III-8. Photomicrograph of control auk let liver stained with Prussian Blue to demonstrate hemosiderosis. Note lack of iron containing pigments. Vacuolar lipid accumulation in liver is common in breeding female birds. 630x.

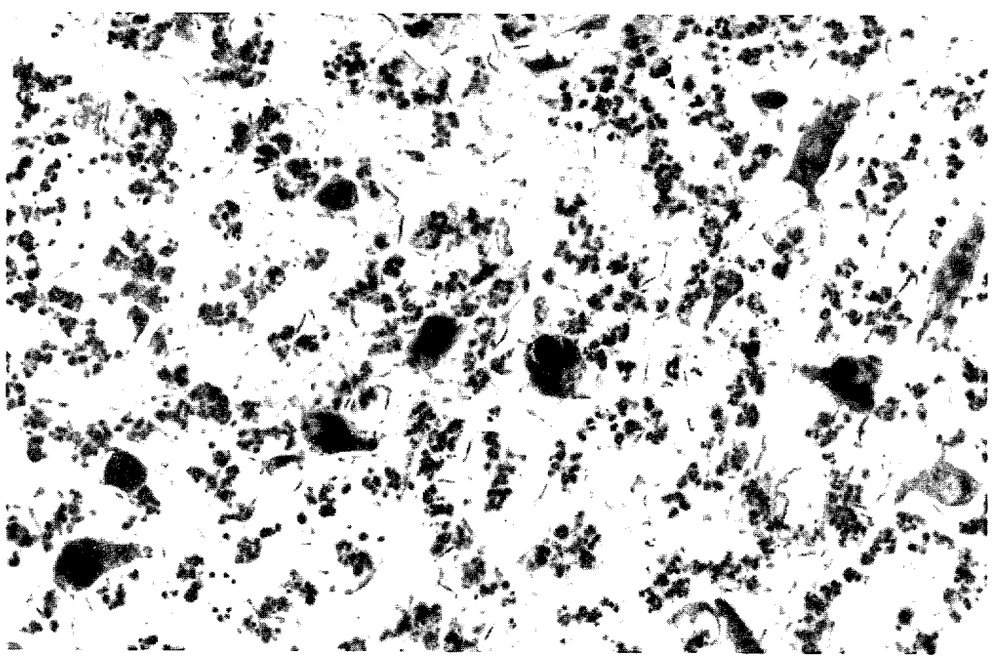


Figure III-9. Photomicrograph of liver stained with Prussian Blue to demonstrate hemosiderosis. Auklet exposed to oil in captivity for four days. Liver is heavily pigmented. Black deposits in hepatocytes and Kupffer cells represent hemosiderin. 630x.

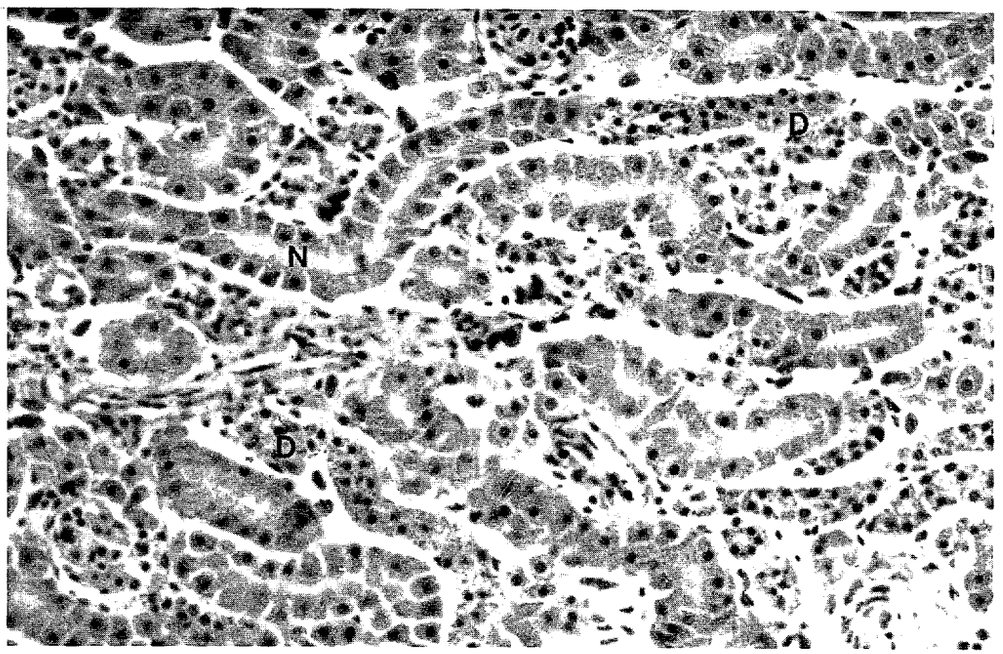


Figure 111-10. Degenerating collecting ducts (CD) adjacent to normal collecting ducts and convoluted tubules within the kidney of an oiled Cassin's auklet. H and E, 340x.

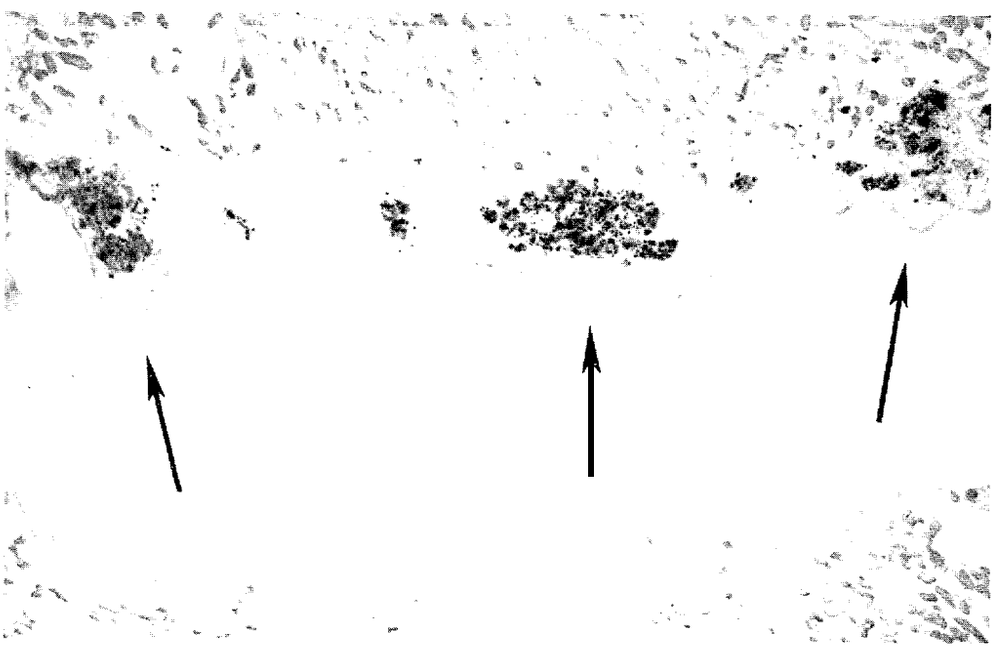


Figure 111–11. Pneumoconiosis in lung of a Cassin's Auklet. Macrophages containing crystalline particulate were present in intraatrial septa of tertiary bronchi (arrows) of both control and oil exposed birds. Prussian Blue Stain, 300X.

Multiple foci of necrosis and inflammation appeared in the upper GI tract in six of the seven murres. In three of these birds, nematodes were present either grossly or histologically (probably Entracecum sp.) (Figure 111-12). In 3 murres, pinpoint reddish grey to tan intestinal nodules were found histologically to be necrotic foci associated with penetration of the mucosa by tape worms (Figure 111-13). Additional parasites included a peritoneal nematode in one bird, coccidial gametes and oocysts in the intestinal epitheliums or lamina propria (Figure 111-14) and unidentified parasitic ova within the ureteral and cloacal mucosa of one bird and in the cloaca in another. Three murres had marked generalized lymphoid hyperplasia of undetermined etiology. Two birds exhibited focal pancreatitis.

Mild renal tubular necrosis was present in four of seven murres. In one of these birds a few urate tophi were present; in another, mineralized tubules were seen. One murre had occasional collecting tubules containing enlarged nuclei with marinated chromatin and basophilic intranuclear inclusion bodies (Figure 111-15). Electron microscopic examination confirmed the presence of adenovirus-like particles (Lowenstine and Fry 1985).

Disassociation of hepatocytes was not seen in the oiled murres. Hemosiderosis of hepatocytes and Kupffer cells was present in 6 of 7 murres (Figure 111-16), but was generally not as marked as in the auklets. Two murres had mild peripheral lymphoplasmacytic hepatitis. One of these and two additional birds had hepatocellular fatty change (Fig. 111-17), a lesion not seen in the auklets.

Grossly, salt glands of four murres were smaller than expected (below the level of the orbit) and a gland of one was grossly lobulated. Histologically, that gland showed fibrosis of the interlobular connective tissue. Another murre exhibited mild focal necrosis of the salt gland accompanied by a few enlarged nuclei with marinated chromatin, possibly of viral etiology. Adrenal size of murres varied considerably. In one female, they measured 7 x 3 x 3 mm. In 5 males, they ranged from 8 x 5 x 3 mm to 11 x 6 x 3 mm (one male was not measured). Microscopically the ratio of cortex to medulla was increased in three of the seven murres.

This preliminary study provided histopathological information on wild alcids exposed to small quantities of weathered Santa Barbara crude or Bunker C oils. Although these oils were of quite different composition, they caused similar toxic reactions, including liver cell disassociation, hemosiderosis, and renal tubular necrosis, but did not result in marked intestinal irritation, adrenal hypertrophy or salt gland hypertrophy.

The captive conditions for auklets were not optimum as evidenced by the refusal of birds to eat, and weight loss of all birds during the first days in captivity. Mild anemias developed in captive controls accompanied by mild iron pigmentation (hemosiderosis) of the liver. This change is not specific for oil intoxication as it was also seen, but to a less severe degree, in control auklets kept in captivity. Hemosiderosis, a common occurrence in some species of birds in captivity may be multifactorial (Lowenstine and Petrak 1980), caused by dietary excesses of iron or hemolytic and aplastic anemias. Hepatocellular damage enhances liver iron storage, as demonstrated by

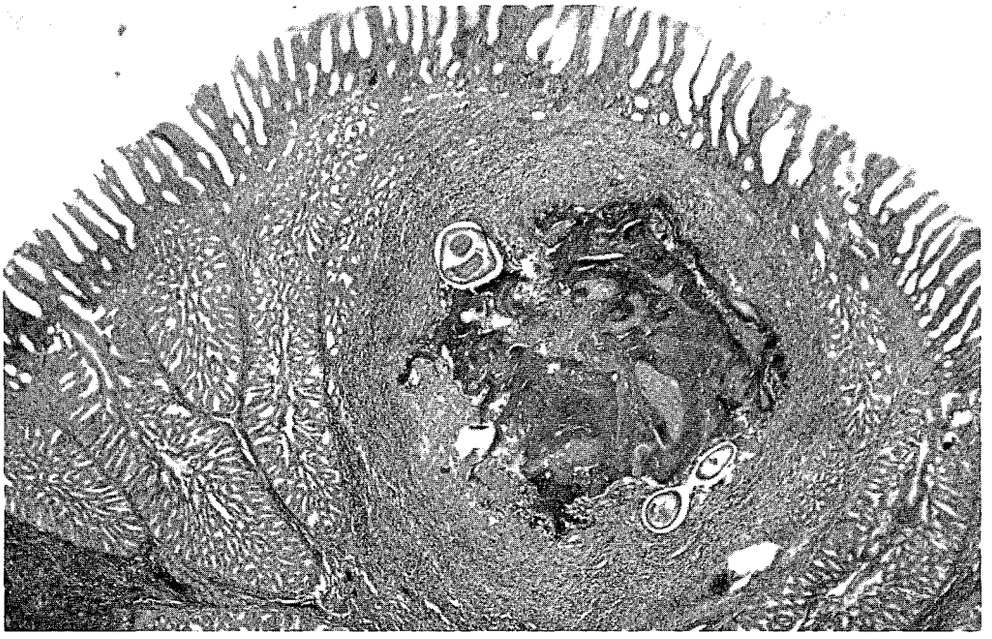


Figure 111-12. Focal necrosis within the submucosa of the proventriculus of an oiled Common Murre. Necrosis is associated with profiles of nematodes and surrounded by granulomatous inflammation. H and E, 60x.

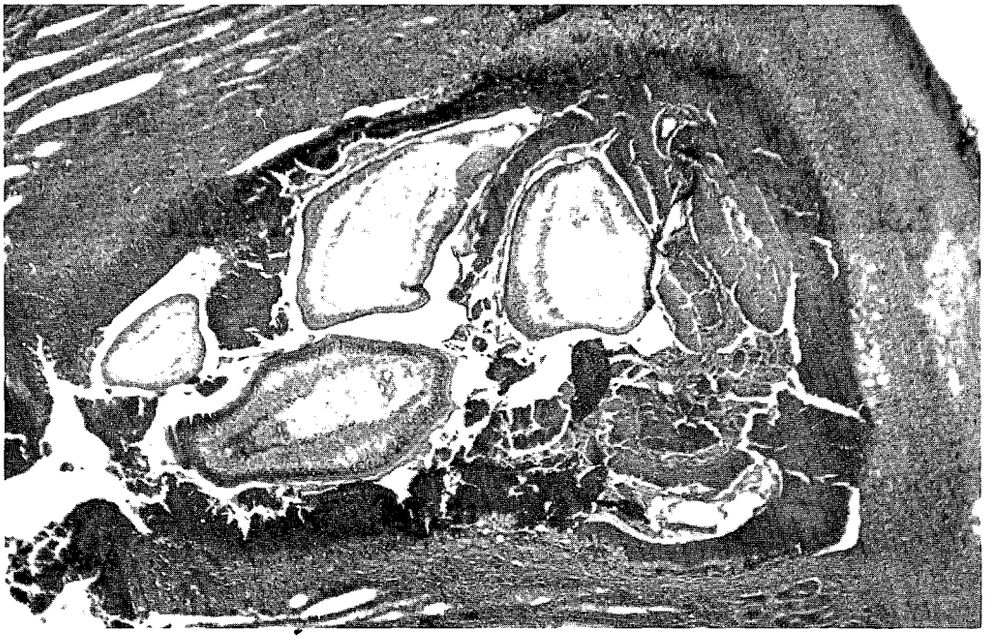


Figure 111-13. Multifocal ulceration and necrosis of the intestine of an oiled Common Murre. Sections of a cestode are embedded in the ulcer. H and E, 50x.

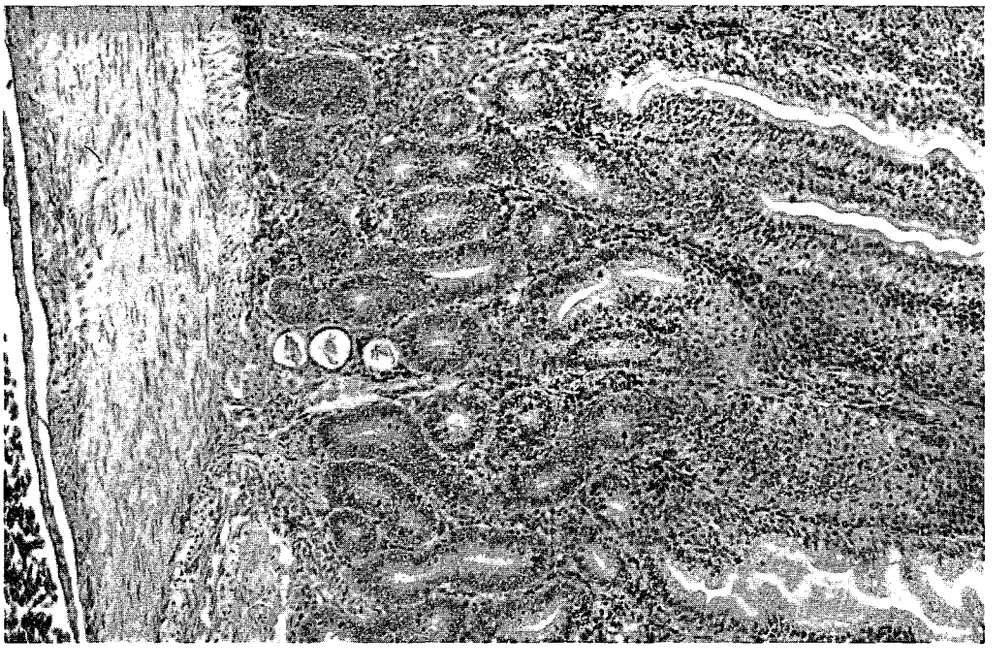


Figure 111-14. Coccidial oocysts (arrow) present in the lamina propria of the intestine of an oiled murre. Coccidial gametes were present in intestinal epitheliums of other birds. H and E, 225x.

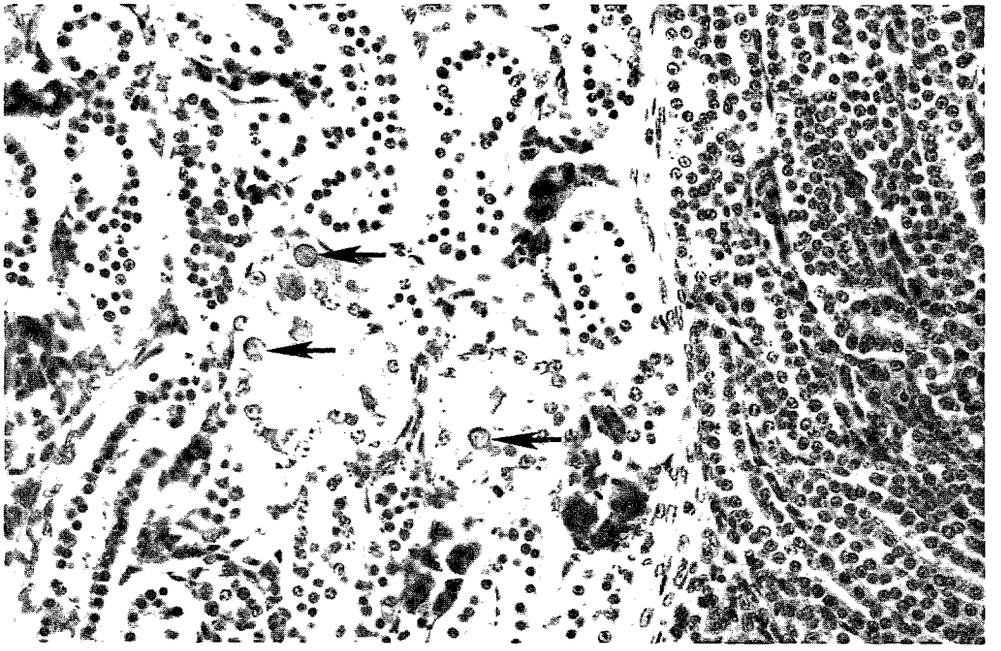


Figure 111-15. Renal tubular degeneration was seen in four murres. Degeneration of tubule with enlarged nuclei with marinated chromatin (arrows) suggests a viral infection. H and E, 240x.

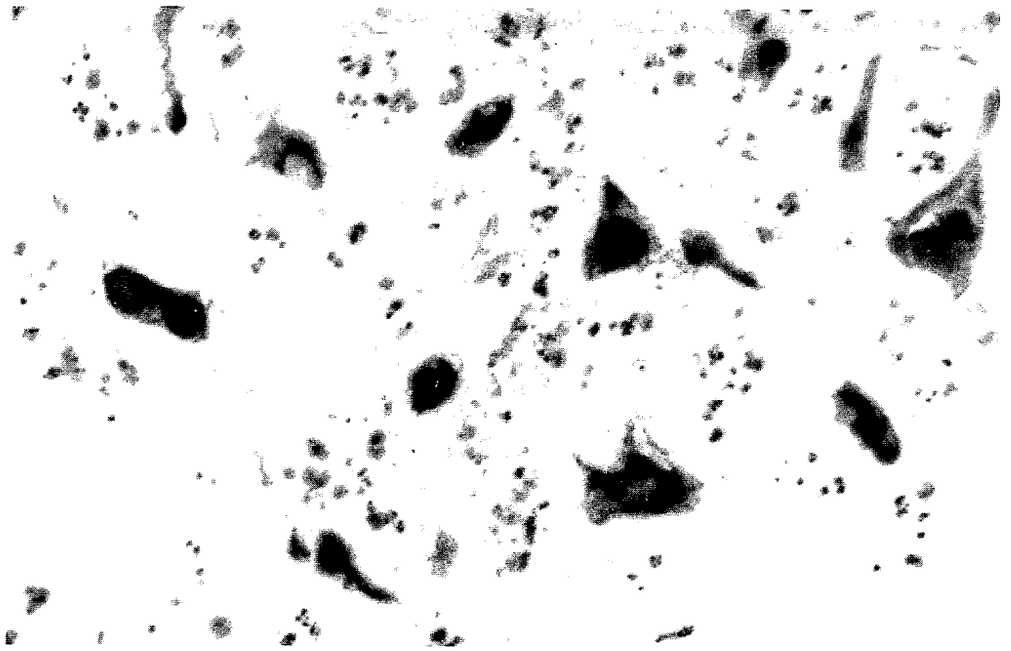


Figure 111–16. Hepa to cellular and Kupffer cell hemosiderosis present in an oiled murre. Prussian Blue stain, 420x.

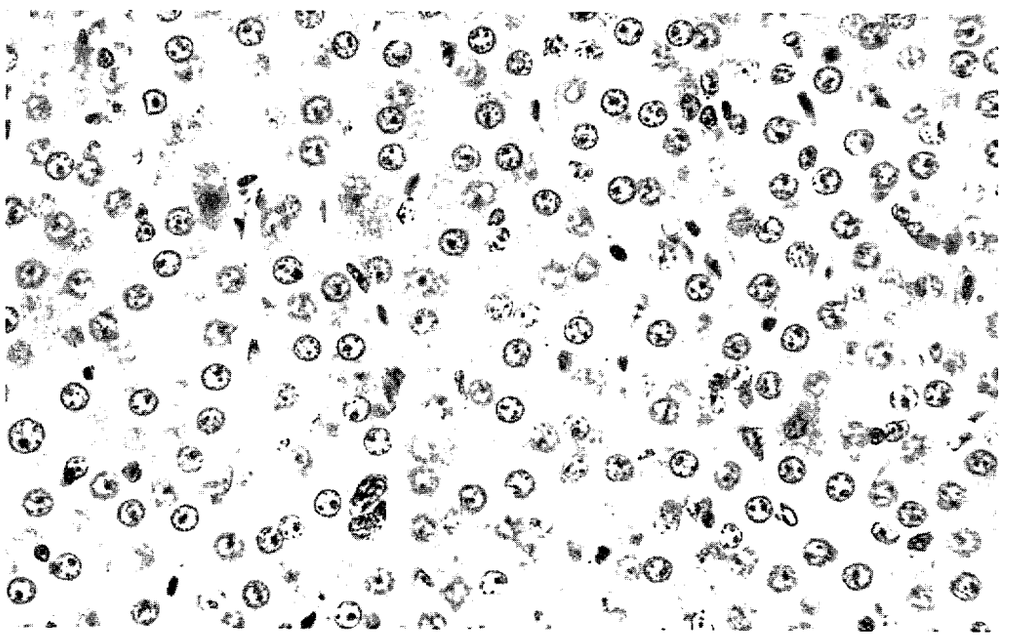


Figure 111-17. Mild hepatocellular vacuolation characteristic of fatty change was seen in oiled murres. H and E, 420x.

experimental lead intoxication (McDonald and Lowenstine 1983). Hepatocellular individualization was observed in all oiled auklets and in the two controls kept in captivity, again to a lesser degree. This change can be precipitated by direct hepatocyte damage or by hypoxia as might occur in an anemic animal.

A markedly depressed hematocrit (21% of blood volume) was measured in the one surviving oil-exposed auklet and mild anemia (hematocrits 8-24% lower than normal) was measured in all oiled murres. The hemosiderosis and Kupfer cell iron pigmentation were additional indications of anemia, although not conclusive on their own. The stress of captivity may have contributed to anemia, liver pigmentation, and disassociation of hepatocytes, but oil exposure markedly increased the severity of these conditions; additionally, it caused renal tubular degeneration not seen in controls.

B. Experimental Study of Heinz Body Hemolytic Anemia in Chickens

The severe anemia observed in captive auklets and oiled murres data and the report of Leighton et al. (1983) led to an investigation of anemia in birds exposed to oil in spills and collected from beaches for rehabilitation. The investigation of hemolytic anemia was initiated with experiments to evaluate the available methods for preparing blood smears to demonstrate Heinz bodies in avian red blood cells.

Anemia was induced in chickens experimentally by injection of phenylhydrazine hydrochloride (PHH) intra muscularly at the dose of 13mg/kg body weight. PHH causes oxidation and dumping of hemoglobin within red blood cells resulting in the formation of Heinz bodies. Heinz bodies were detected by staining either fresh or dried blood smears with Crystal Violet (Methyl Violet 10B) or new Methylene Blue. A more satisfactory method, but more difficult to perform in the field, was to mix fresh blood with dilutions of brilliant green and neutral red (Leighton et al. 1983).

None of the light microscopic methods was very satisfactory for reliable demonstration of Heinz bodies, as avian Heinz bodies are smaller than those induced in mammalian red blood cells and do not stain prominently. Cells with Heinz bodies were more easily demonstrated by scanning electron microscopy (SEM), even in unstained smears.

1. Scanning Electron Microscopy (SEM)

Blood cells were prepared for SEM by cutting microscope slides of air dried blood smears with a diamond pencil into .5 cm pieces and mounting them on an SEM stub. Stubs were coated with approximately 10 nm of gold and viewed with an 1S1 scanning electron microscope equipped with a tungsten filament operated at an accelerating voltage of 10 KV. Cells were evaluated for surface membrane irregularities.

Normal, untreated red cells are flattened elipsoids. The surface tension of drying causes tight adherence of the cell membrane so that the underlying nuclei are often outlined beneath the cell membrane which tightly conforms to the underlying structures. The cell membrane overlying hemoglobin in normal cytoplasm was very smooth, reflecting the homogeniety of the hemoglobin (Figure 111-18). Dried smears taken from experimentally anemic birds were markedly different in that the cells have membranes overlying uneven cytoplasm, demonstrating precipitation of hemoglobin in affected cells (Figure 111-19).

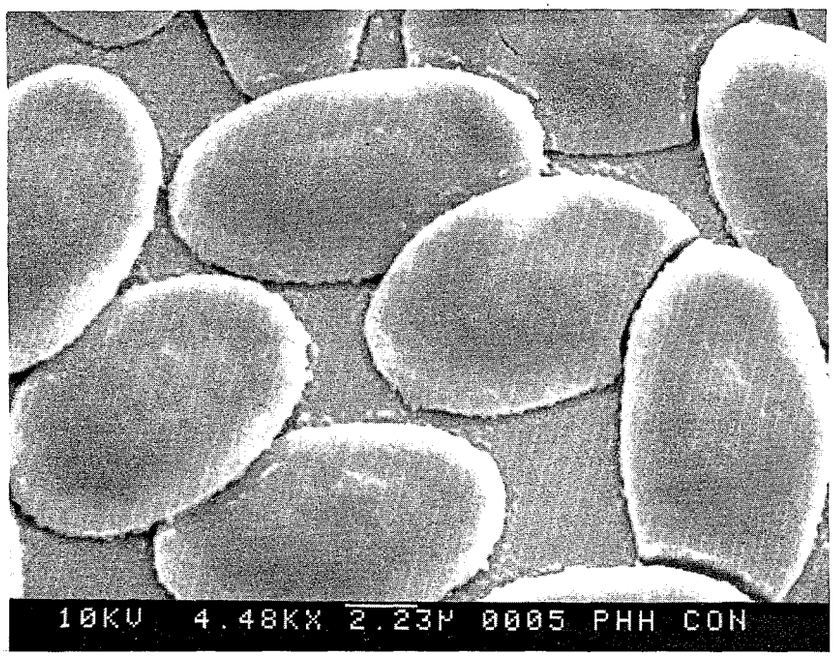


Figure III-18. Scanning Electron Micrograph of blood cells of a control chicken 4,480 x.

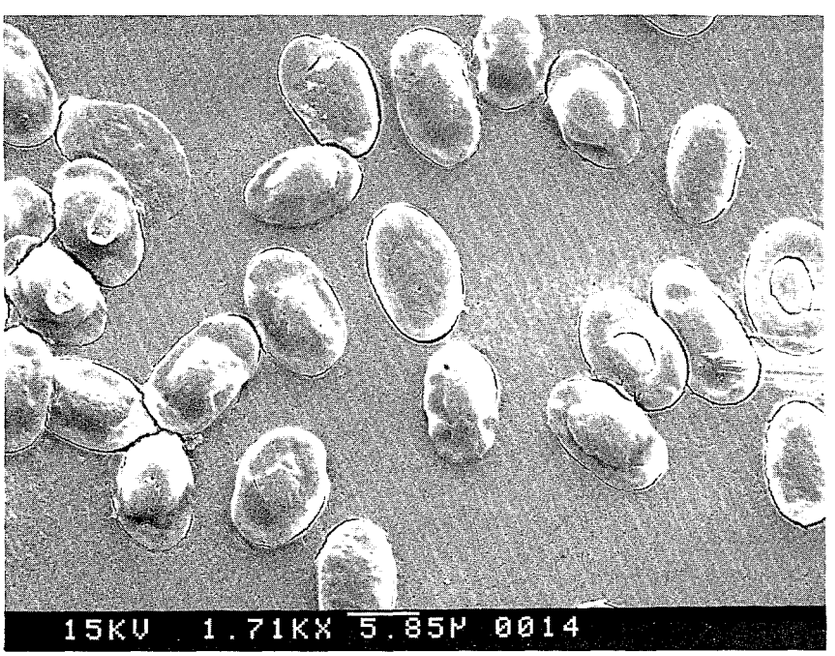


Figure 111-19. Scanning Electron Micrograph of blood cells of a chicken exposed to Phenylhydrazine hydrochloride. Day 1 after exposure. 1,710 x.

In experimentally induced anemia of chickens, the hematocrits were only minimally reduced even though the morphology of a large percentage of the red cells was altered. Recovery of red cells was evident over the course of 4-6 days. At one day after treatment most of the red cells exhibited hemoglobin precipitates. At four days, a majority of the cells showed a slightly uneven cell surface indicating some inhomogenei ty of hemoglobin, but the extent was less severe than on day one. On day six, most cells had smooth or only very slightly uneven contours (Figure 111-20).

The large proportion of cells affected on day one were not removed from the circulation as the hematrocrit did not significantly fall during the experiment. Furthermore, the uniform morphology of the red cells in the circulation, and the reduced extent of damage at four and six days indicated that the affected cells recovered rather than damaged cells having been removed from the circulation and replaced by new red cells or reticulocytes.

The possibility of recovery of red cells rather than removal from the circulation has not been reported in the literature. Not all agents which induce Heinz bodies, including petroleum products with a large polynuclear aromatic component, act similarly. The petroleum-altered red cells observed were removed from the circulation with a resulting drop in hematocrit. The mechanisms of the different responses to PHH and petroleum are unexplained.

C. Studies of Blood Cells of Murres Exposed To Oil In Ocean Spills

An unidentified vessel discharged a relatively small amount of Bunker C fuel oil off the coast of San Mateo and Santa Cruz Counties, CA, in June 1983. Approximately fifty oiled murres beached themselves near Ano Nuevo Point and were taken by volunteers to Santa Cruz, CA. Blood was collected for hematocrit determinations and for preparation of blood smears. Smears were examined by SEM to compare Common Murre and chicken blood. The SEM was used to determine whether Heinz bodies were present and similar to those produced experimentally in the red blood cells of chickens. Figures 111-21 and III-22 are examples of the presumed normal and Heinz body-containing red cells taken from oiled murres. In these murres, only a small proportion of the cells contained Heinz bodies 3-4 days after the birds were cleaned. The hematocrit of the murres was markedly reduced, however, and many affected cells had probably already been removed from the circulation. Hematocrits of 8 murres examined ranged from 14-32% with an average of 22%, representing a decrease of 40-75% of the circulating red blood cells.

D. Studies of Blood Cells of Oil Dosed Auklets

Blood samples were obtained from 5 control auklets and 7 auklets dosed externally or orally with 1 ml weathered crude oil as part of the 1984 field study on SEFI. Hematocrit values of 3 externally dosed birds 6 days post dosing and 4 orally dosed birds 6 and 7 days post dosing were not significantly different from control values (See Table III-2 below).

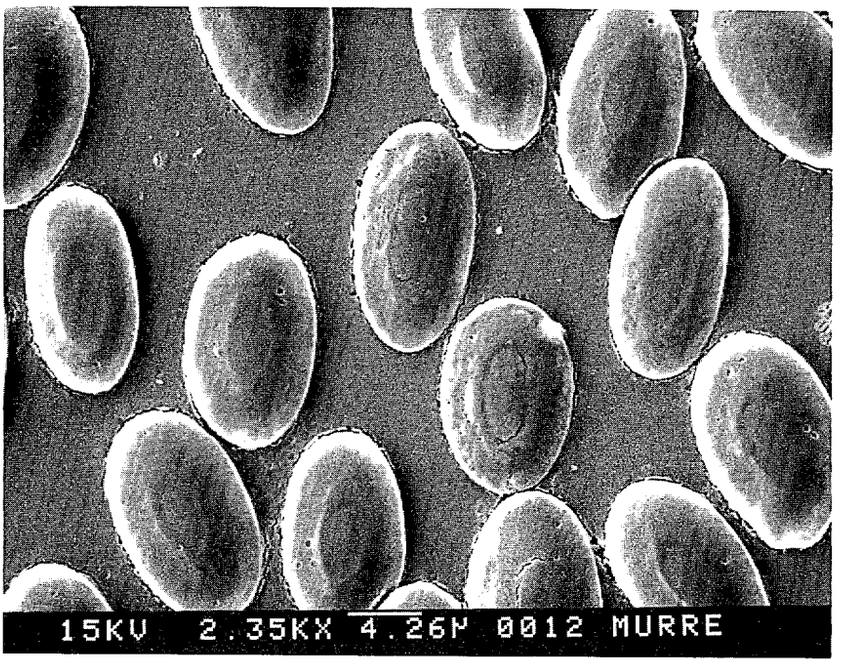


Figure 111-21. Scanning Electron Micrograph of blood cells of a Common Murre exposed to Bunker C. Morphology of many normal appearing cells. 2,350 x.

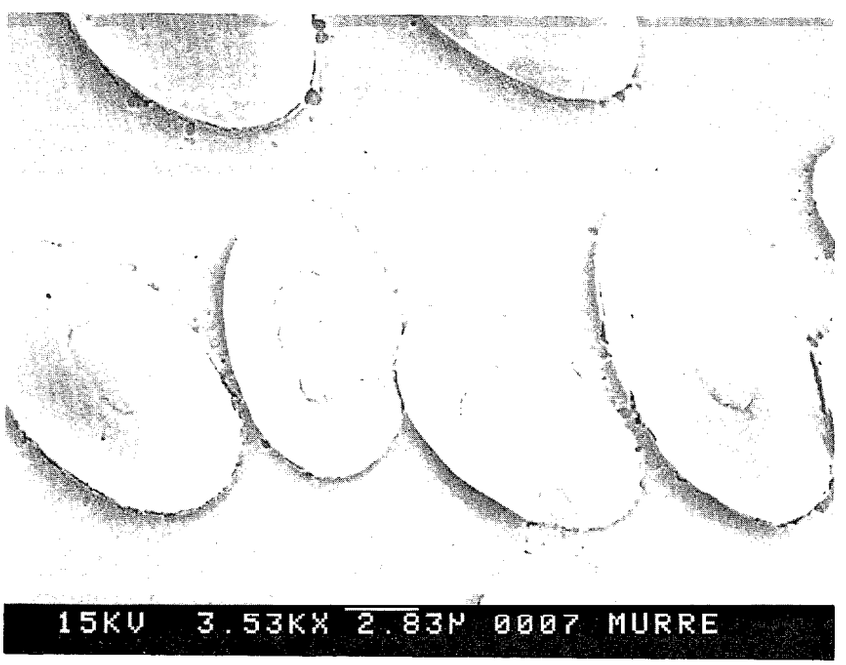


Figure 111-22. Scanning Electron Micrograph of blood cells of a Common Murre exposed to Bunker C. Morphology of abnormal cells containing precipitates of hemoglobin (Heinz Bodies). 3,530 x.

TABLE III-2. Hematocrit Values of Cassin's Auklets Experimentally Oil Exposed in the Field

		MEAN
Control	59, 53, 54, 52, 58, 36*	52
External Dose	58, 46 (one lost)	52
Oral Dose	48, 52, 50, 52	50

*One control appeared to be anemic for unknown reasons.

A single blood smear from each bird was examined by SEM to determine surface morphology of cells. One externally dosed bird showed mild red cell changes consisting of irregular surface texture and evidence of aggregates of hemoglobin beneath the cell surface. Three of four orally dosed birds also exhibited small aggregates on the margins of red blood cells (Figures 111-23, 111-24) and small protrusions similar to the pinching off of membrane blebs for extrusion of Heinz bodies as described by Jain and Keeton (1975) in hemolytic anemia in cats.

The changes appeared to be mild and although they probably indicated only minimal damage to the overall oxygen carrying capacity of the blood, they are a sensitive indicator of oil toxicity.

E. Studies of Murres Oiled in the Spill from the Tanker "Puerto Rican"

On October 31, 1984 the tanker "Puerto Rican" exploded 12 miles outside San Francisco Bay. Rough seas broke the ship apart on November 3, and the stern section sank in 350 fathoms of water 11 miles south of SEFI. The accident spilled an estimated 35,000 barrels of mixed oils and oil additives including 11,730 barrels of Witco 2033TR, a lubricating oil, 9,348 barrels of OLOA-246B, a proprietary oil additive, and 4,614 barrels of polybutene. Additionally, up to 16,500 barrels of mixed alkanes (identified by the shipper only as Alkane 56 and Alkane 60) were in cargo tanks damaged by the explosion, but the amount of loss of this cargo was unclear as water may have displaced part of the cargo in some tanks. The fuel tanks in the stern section were nearly full and contained 8,500 barrels of No. 6 fuel oil ("Bunker C"), which began leaking and rising to the surface when the pressure crushed the tanks. Four thousand gallons of Corexit 9527, a dispersent, were applied by an aerial tanker to the major oil slick on November 3, but was largely ineffective in dispersing the refined lubricating oil and oil additive.

By November 8, the oil slick had drifted north towards the Point Reyes coastline. Birds caught in the spill included Common Murres, Western Grebes, Common Loons, and Surf Scoters (Page and Berkner, 1985). Oiled birds were taken to Fort Cronkite, Marin County, where a temporary rehabilitation center was located. Birds brought to the center were banded, weighed, given fluids to reduce dehydration, and placed in a warm and dry environment. Approximately 215 birds were rehabilitated and released, while an estimated 1,100 birds died in rehabilitation centers. An additional 1,300 dead birds were collected from beaches by volunteers (Page and Berkner, 1985).

Hematocrits were determined for more than 40 birds brought to the rehabilitation center. Most were below normal and remained low throughout

Figure III-23. Scanning Electron Micrograph of blood cells of a control Cassin's Auklet. 2,230 x.

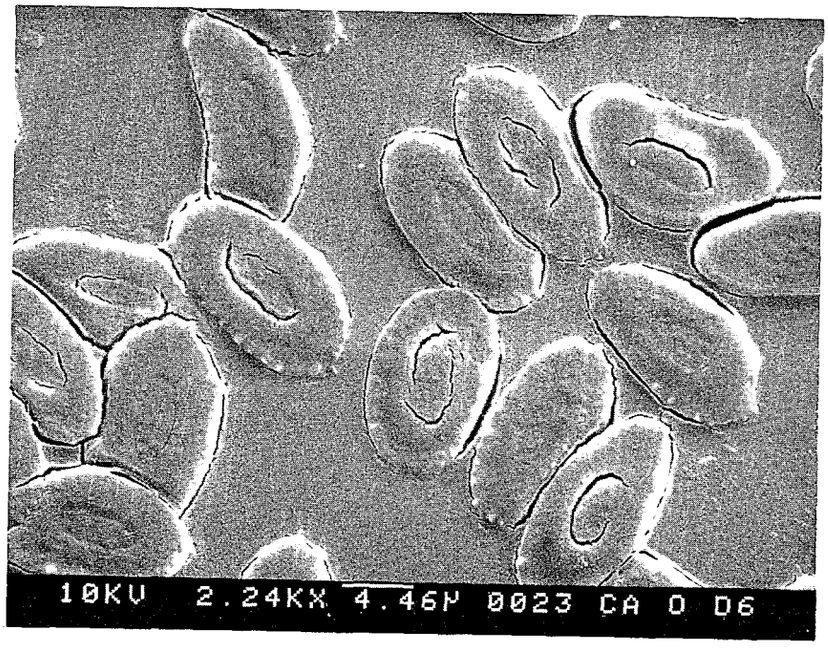


Figure III-24. Scanning Electron Micrograph of blood cells of a Cassin's Auklet oral dosed with 1 ml weathered Santa Barbara crude oil. *Note* protrusions on *the* margins of blood cells. 2,240x.

the study period. A group of 8 murres was monitored throughout the rehabilitation process to determine the progression of anemia. Blood samples were taken from the tarsal vein on alternate days beginning 2-3 days after oiling. Hematocrit determinations and blood smears were prepared for WBC analysis and SEM.

Two murres kept in captivity for one month after cleaning showed no improvement in hematocrit. Suboptimal captive conditions, residual effects of oil or stress may have contributed to such a long term lack of a regenerative red blood cell (RBC) response.

1. White Blood Cell (WBC) Analysis

Total WBC counts and WBC differentials were performed at UCD. WBC differential slides were stained with Wright's Stain and the percentage of each cell type was determined by counting 100 cells. Total WBC counts were calculated from dried blood smears (Table III-3). The percentage of heterophils was markedly elevated and that of lymphocytes depressed 2 days after oiling. Both cell types remained altered for the duration of captivity. Monocytes and eosinophils remained above normal, while basophils stayed within the normal range. Total WBC numbers declined during captivity.

The changes in WBC types, which included increased numbers of heterophils and reduced lymphocytes, are typical of responses to toxicity or acute infection, and may be adaptive for birds at increased risk. Oiled murres, however, experienced a decrease in total WBC count throughout the study period which would eventually reduce the bird's ability to combat infection or toxicity.

Toxicity from ingesting oil during preening, cold stress from matted wet plumage, and captivity all contributed to the stress of oiled birds. Stress causes a rapid increase in heterophils and an accompanying decrease in lymphocytes, which can be induced by capture, handling, and transport of Common Murres (Bradley and Threlfall 1974). All of the oiled murres showed typical WBC responses to stress which may be mediated by the adrenals. The responses were similar to reports of administration of corticosterone or corticotrophic drugs resulting in an increase in heterophils in chickens (Newcomer, 1959; Davison and Flack, 1981).

2. Analysis of Oil Removed from Feathers of Beached Murres

Samples of cargo products and bunker fuel from aboard the "Puerto Rican" were obtained from the U.S. Coast Guard for analysis. Oiled feathers were collected from experimental birds at the time of the first blood sampling (2-3 days after oiling).

Oil from murre feathers was identified by comparing distillation profiles and mass fragment patterns with those of the cargo products. The methods and analysis are presented in Section IV. All feathers were found to be contaminated with Bunker C fuel oil or mixtures of Bunker C and OLOA-246B. Additionally, two of eight murres were contaminated with oil containing the dispersent Corexit 9527. The total amount of Corexit 9527 on oiled feathers appeared to be very low.

F. Discussion of the Acute Toxic Effects of Oil on Alcids

The literature describing oil toxicity has been confusing, partly because many toxic components have not been identified and compositional

TABLE III-3. WHITE BLOOD CELL DIFFERENTIAL VALUES FOR OILED MURRES

DAYS POST OIL		HETERO %	LYMPHO %	MONO %	EOSIN %	BASO %	WBC %
2-3	MEAN	71.3	6.5	9.8	11.1	3.5	8.3
	SEM	4.0	1.9	1.4	2.8	0.8	1.3
	N	11	11	11	10	8	11
4-5		77.8	6.3	10.1	3.3	3.5	7.7
		2.3	1.4	1.4	2.5	0.6	0.8
		10	10	10	9	8	10
6-7		78.0	7.2	9.4	5.6	2.0	7.6
		3.0	1.3	0.8	1*9	0.3	0.8
		10	10	10	8	7	10
8-9		64.9	13.2	12.9	7.9	2.4	6.0
		5.3	2.8	3.4	2.3	0.3	0.6
		10	10	10	8	7	1 0
10-11		67.3	10.3	15.7	6.2	1.7	5.3
		4.1	3.1	2.3	0.9	0.3	0.4
		7	7	7	6	6	7

differences between oils result in different symptoms. Additionally, interpretation of some symptoms is difficult because of the additive effects of oil toxicity on birds already stressed and in poor condition. The significance of information describing the histopathology of normal auklets goes beyond simply providing control information for the present oil study. Few data exist in the literature to help determine whether or not the tissues of a seabird are normal. From the above observations, it is clear that the presence of a variety of parasites, gut lesions and pneumoconiosis are common, and would not, of themselves, indicate that a bird was in poor condition from exposure to oil or other pollutants.

Birds exposed to oil may suffer injuries at a variety of levels: (a) immediate external effects; (b) secondary toxicity arising from ingestion of oil with resultant physiological changes and compensatory responses of other tissues; and, (c) long-term effects manifested in exposed adults, chicks exposed to oil or fed contaminated food, and in chicks hatched from eggs of exposed birds.

Immediate effects include fouling of plumage, subsequent wetting of the bird, loss of buoyancy, and inability of the bird to fly or forage. These primary physical effects are common to most viscous petroleum products and are not related to the toxicity due to specific chemical components. Fouling and wetting of plumage causes increased thermoregulatory demands with the consequence of slightly increased basal metabolic rate (Hartung 1967, McEwan and Koelink 1973, Lambert et al. 1982). It is likely that oil fouling and cold stress from wetting are responsible for the majority of seabird losses in an oil spill.

Ingestion of toxic substances during preening results in secondary effects. Intestinal irritation by refined (No. 2) or residual (No. 6) fuels oils may cause bleeding into the gut lumen (Hartung and Hunt 1966, Langenburg and Dein 1983). Hemorrhage and bloody stools were not observed with any auklets or murres, but scoters and other ducks appear to be particularly susceptible (Hartung and Hunt 1966, Langenburg and Dein 1983). Naphthenic crude oils with high aromatic and asphalt content may cause intestinal mucosal damage in alcids as well as waterfowl, including impairment of water and sodium transport with accompanying dehydration and salt stress (Crocker et al., 1974, 1975; Miller et al 1978).

Liver and kidney damage have been reported as secondary effects of oil intoxication. Renal focal necrosis resulting from exposure to No. 2 (diesel) fuel oil or South Louisiana crude has been described by Howard et al (1979) and Szaro et al (1981). Hepatic degeneration and fatty infiltration of the liver were found in studies by Hartung and Hunt (1966), Szaro et al. (1981), and Langenburg and Dein (1983) when ducks were exposed to No. 2 or No. 6 (bunker) fuel or other refined oils. Hepatic mixed-function oxidase is induced and hepatocyte degeneration is evident from elevation of serum liver enzymes Szaro et al. 1981, Patton and Dieter 1980, Gorsline and Holmes 1981, 1982a, b).

In the present study, fatty changes were observed in the one female and two male murres. The fatty changes occurring in the female may have been incidental to breeding as female birds develop fatty livers during yolk synthesis and mobilization, which complicates toxicological interpretation.

Absorption of oil with a high polyaromatic hydrocarbon content (some crudes from South Louisiana, Santa Barbara, and Prudhoe Bay, as well as residual oils such as Bunker C) results in precipitation of hemoglobin, producing a Heinz body-type hemolytic anemia (Leighton et al. 1983). The hemolytic crisis takes place between 3 and 6 days post exposure. Recovery began by day 7, but birds were killed before recovery was complete. In this study, murres in rehabilitation centers did not recover from anemia, even when force fed a high calorie diet in addition to fish ad libitum and supplementing the diet with iron dextran injections to stimulate blood cell regeneration.

The lack of recovery may have been partly a consequence of stress, as alcids are difficult to maintain in captivity even when healthy. The non-specific stress of oil fouling and the physiological stress of oil toxicity could intensify adverse reactions. The WBC changes observed in oiled murres were also consistent with a response to stress and infection.

Normal alcid WBC differential profiles of alcids obtained from the literature are combined in Table III-4. In general, heterophils are the most abundant cell type seen in these species. In most other avian species lymphocytes predominate. There is much variation in the reported values in the literature, perhaps due to captivity of some birds or differences in staining techniques of white blood cells which result in different ratios of heterophils and granulocytes.

Captivity is a known stressor for wild birds. Bradley and Threlfall (1974) documented a rapid increase in heterophils and an accompanying decrease in lymphocytes induced by capture, handling, and transportation of Common Murres. Blood samples taken in the field were approximately 23% higher for lymphocytes and 27% lower for heterophils than samples taken in the lab. In domestic chickens, restraint has been shown to be a stressor by inducing marked heterophilia (Besch et al. 1967; Newcomer, 1958). Additionally, chickens that were administered corticosterone injections or drugs which increase corticosterone showed an increase in heterophils (Newcomer, 1958; Davison and Flack, 1981).

Murres in the present study were subjected to a great deal of stress, not only from oil exposure, but also from capture, transport, housing in cleaning centers, cleaning, and force feeding. These additional stresses may have been responsible for the abnormal WBC counts. It is impossible to determine whether the additional trauma of being oiled had an effect on WBC types and numbers.

Oil exposure in experimental studies has also demonstrated changes in adrenal function which may be partly compensatory to stress as well as direct toxic effects. South Louisiana crude (pump date 1976, a high aromatic crude identified as SLC-76) studied by Miller et al. (1978) and Peakall et al. (1981) produced adrenal hypertrophy and short-term increases in circulating corticosterone in Herring Gulls, which were interpreted as a response to acute stress resulting in increased in pituitary corticotrophin and adrenal corticosterone secretion. Chronic feeding of Prudhoe Bay crude, South Louisiana crude (API ref. oil) or North Sea crude to ducks carried out respectively by Rattner and Eastin (1980), Gorsline and Holmes (1982a, 1982b), and Harvey et al. (1981) all resulted in long-term decreases in circulating corticosterone. The decreases in serum corticosterone were accompanied by increases in

corticotropin and adrenal hypertrophy, but direct adrenal impairment resulted in reduced ability to synthesize or release corticosterone (Gorsline and Holmes, 1982a, b). Decreases may have been further compounded by metabolism of adrenocorticoids following induction of mixed-function oxidases in the liver. The complexity of the toxic response was demonstrated by Gorsline and Holmes (1982b), when distillation of South Louisiana crude into four boiling fractions separated the liver enzyme induction effects from the adrenal inhibitory effects. The responsible compounds in each distillate fraction were not identified.

Stress may be further compounded by toxic effects of oil on electrolyte regulation and salt gland function. Hypertrophy of the salt gland has been induced by high aromatic crudes such as South Louisiana (SLC-76)(Peakall et al. 1981, 1983), and Prudhoe Bay (or a polynuclear aromatic fraction isolated from Prudhoe Bay crude) (Peakall et al. 1982), and by Kuwait crude (Miller et al. 1978). The hypertrophy may have been compensatory to impaired function, and mediated through increased adrenocorticoid output.

Salt gland function in seabirds is influenced by adrenocorticosteroid secretion and by increased plasma sodium (Peakall et al. 1980). The complex interactions between intestinal sodium and water transport, adrenal impairment with compensatory hypertrophy, and direct salt gland inhibition make electrolyte balance and dehydration significant stresses for oiled seabirds. The sum total of the additive effects of oil, salt stress, and cold have been experimentally induced in ducks by Holmes et al. (1979) with the consequence of increased mortality.

TABLE III-4. NORMAL ALCID WHITE BLOOD CELL DIFFERENTIAL VALUES

SPECIES	HETERO[1] %	LYMPH %	MONO %	EOSIN %	BASO %	WBC # x 1000
RAZORBILL (Alca torda)						
Ref: Bradley and Threlfall (1974)	37	41	5	11	0	
Ref: Nikitenko (1965)	65	25	2	5	4	
THICKBILLED MURRE (Uris lomvia)						
Ref: Bradley and Threlfall (1974)	25	61	4	8	2	
Ref: Nikitenko (1965)	49	33	3	10	5	
COMMON MURRE (Uris aalge)						
Ref: Bradley and Threlfall (1974)	18	52	4	19	5	
Ref: Nikitenko (1965)	64	29	2	3	4	
BLACK GUILLEMOT (Cepphus grylle)						
Ref: Bradley and Threlfall (1974)	4	32	3	59	0	
Ref: Nikitenko (1965)	60	30	0	4	3	
PIGEON GUILLEMOT (Cepphus columba)						
Ref: Calambokidis, et al. (1985)	SO	44	3	0.6	1.7	5
CASSIN'S AUKLET (Ptychoramphus aleuticus)						
Ref: Fry and Lowenstine (1985)	77	21	2	0	0	7
COMMON PUFFIN (Fratercula artica)						
Ref: Bradley and Threlfall (1974)	22	59	3	15	1	
Ref: Nikitenko (1965)	58	29	3	5	5	

[1]: Abbreviations: HETERO: Heterophils; LYMPH: Lymphocytes; MONO: Monocytes; EOSIN: Eosinophils BASO: Basophils; WBC: Total White Blood Cell Count

IV. OIL ANALYSIS

A. Methods

1. Analysis of Crude Oil and Oil Residues in Contaminated Tissues

Analyses of both fresh and 6-day weathered crude oil were performed using methods comparable to those of Hallett et al. (1983). Crude oil was dissolved in dichloromethane and separated into aliphatic, aromatic and polar fractions on an activated silica gel column (dried in a 200°C. oven for 12-14 hr) by successive elutions with n-hexane, n-hexane: benzene (1:1), and methanol :dichloromethane (1:1). The fractions were evaporated to dryness, redissolved in benzene or dichloromethane, separated by gas chromatography (GC) with a 30-meter glass capillary column (J&W Scientific, DB-5), using a temperature program from 50 to 275*C at 3.5°C/min. Major peaks were identified by mass spectroscopy (MS), and peaks not identified by MS were quantified by integration of the chromatogram and reported as resolved hydrocarbons.

Analyses of oil residues in auklet tissues were made at UCD using methods similar to those of Gay and Belisle (1980), and Belisle et al. (1981) by homogenization in 4N KOH, saponification by refluxing 3 hours at 90°C, and elution from a partially deactivated (3% water by weight) Florisil cleanup column with 6% diethyl ether in pentane. Aliphatic and aromatic fractions were separated on a neutral alumina (Fisher Scientific) column with hexane followed by 4:1 benzene:hexane. No polar fraction was collected as the analysis performed indicated weathering removed most polar components.

2. Analysis of Oil Residues from Feathers of Oiled Murres

Two major problems were presented for the analysis of petroleum products contaminating the plumage of beached birds brought to cleaning centers. The small quantities of oil present on feathers precluded the initial cleanup and separation into aliphatic and aromatic fractions employed for analysis of fresh and weathered crude. Additionally, weathering of the products on birds appeared to reduce the volatile fractions of the petroleum. This reduced the total fraction of hydrocarbons which could be separated by the GC within the 300° c maximum operating temperature of the glass capillary column .

Samples of cargo products and bunker fuel aboard the “Puerto Rican” were obtained from the U.S. Coast Guard for analysis. Analysis of products spilled by the tanker “Puerto Rican” was particularly difficult as two of the products were a lubricating oil (WITCO 2033TR) and a proprietary lubricating oil additive (OLOA 246B) composed of middle distillate fractions having boiling ranges of 300-450°C. Less than 5% of the fresh samples of these products were recoverable from the GC with the normal temperature program of 90-270°C. The effect of weathering was to further reduce the fraction which could be separated.

Oiled feathers were collected from experimental birds at the time of the first blood sampling (2-3 days after oiling). The oil was dissolved from the feather with dichloromethane, and under vacuum

to concentrate the residues. A 0.1 ug sample of oil residue was introduced by a temperature controlled probe directly into the high vacuum port of the detector of a Finnegan quadrapole mass spectrometer and distilled off the probe at high vacuum (10^{-5} torr) over the temperature range of 30-250°C.

B. Results

1. Oil and Tissue Residue Analysis

The analysis and comparison of fresh and weathered Santa Barbara channe 1 crude oil is presented in Tables IV-1, IV-2, and IV-3. Total resolved hydrocarbons comprised 141.51 mg/g of the fresh crude oil, while identified peaks comprised only 76.71 mg/g oil. A substantial fraction of Santa Barbara crude has a boiling range above 300°C and could not be eluted off the glass capillary column. The unresolved "envelope" of many different hydrocarbons is characteristic of crude oils and was present in chromatograms of tissue samples of oiled birds as well as in the original oil samples.

Weathering altered the composition of the oil causing a loss of volatile and water soluble components. The relative enrichment of long chain aliphatics, polynuclear aromatics and asphaltenes resulted in a reduced ability to resolve and identify individual compounds. The total resolved hydrocarbons comprised 41.68 mg/g of weathered oil, and identified peaks were only 26.95 mg/g oil. The separation and identification of less than 5% of the total hydrocarbons clearly demonstrates the inadequacy of current gas chromatography (GC) technology for analysis of weathered petroleum.

Chromatograms of the aliphatic residues extracted from the pectoral muscles of auklets are presented in Figures IV-1 and IV-2. Extracts of 0.4 g of muscle were injected into the gas chromatography with a sample split ratio of 1:15. The chromatograms therefore represent the analysis of residues from 27 mg of muscle. The gas chromatography -mass spectroscopy indicates that oil contaminants in tissues can be detected and identified from birds given a single external dose of weathered crude oil. The muscle tissue contamination resulted from ingestion during preening *as* birds removed the oil.

2. Analysis of Products Spilled from the Tanker "Puerto Rican" and Oil Residues from Feathers of Murres

Each of the cargo and bunker products had a distinctive distillation profile; these are presented in Figures IV-3, IV-4 , and IV-5. Mixtures of cargo and bunker oils produced identifiable intermediate profiles. Small amounts of Corexit 9527 were mixed with oils (1:10 and 1:50) and distilled at high vacuum. Corexit could be identified in distillates by a characteristic mass-fragment of mass 211 which was present above background levels only in samples containing Corexit. This fragment, apparently not a molecular ion, probably represents the nucleus of one of the non-ionic surfactants present in the dispersant. The surfactants present in Corexit 9527 are: ethoxylated sorbitan mono-and trioleates, sorbitan monoloeate, and sodium dioctyl sulfosuccinate (Tetra Tech, Inc. 1985). The exact identity of the 211 mass fragment has not been confirmed.

Oils on murre feathers were identified by comparing distillation profiles and mass fragment patterns with those of the cargo products, Corexit, and cargo mixtures. All feathers were found to be

TABLE IV-1. Aliphatic Fraction of Santa Barbara Fresh and Weathered Crude Oil

Compound	Concentration (mg/g) Fresh Crude Oil	Weathered Crude Oil
nC-7	2.11	--
nC-8	2.89	--
nC-9	4.08	--
nC-10	3.68	--
nC-11	3.62	--
nC-12	3.23	0.31
nC-13	3.18	1.05
nC -14	3.24	1.34
nC-15	3.58	2.00
nC-16	2.83	2.34
nC-17	2.12	1.35
Pristane	0.75	0.45
nC-18	2,39	1.43
Phytane	2.26	1.35
nC-19	1.88	1.20
nC-20	2.28	1.42
nC-21	1.91	1.25
nC-22	1.81	1.34
nC-23	1.59	1.16
nC-24	1.49	1.05
nC-25	1.29	0.96
nC-26	1.01	0.88
nC-27	1.36	0.99
nC-28	0.74	0.75
nC-29	0.63	0.59
nC-30	0.50	1.09
nC-31	0.52	0.84
nC-32	0.48*	0.54
Dimethyloctane	1.91	--
Ethylmethylheptane	1.93	--
Dimethylnonae	0.67	--
Methylundecane (2)	0.86	--
Dimethylundecane (2)	0.62	--
Methyldodecane	0.32	--
Methyltridecane	1.57	--
Dimethyldodecane	0.46	--
Dimethyltridecane	0.10	--
Methyloctodecane	0.19	--
Total even n-alkanes	26.57	12.50
Total odd n-alkanes	27.87	11.41
Total n-alkanes	54.44	23.91
Total resolved hydrocarbons	111.16	37.09
Total unresolved hydrocarbons	48.42	83.66
Total hydrocarbons	159.58	121.75
Resolved/unresolved hydrocarbons ratio	2.30	0.44
Odd/even n-alkane ratio	1.05	1.10
Pristine/nC-17 ratio	0.35	0.33
Phytane/nC-18 ratio	0.95	0.94
Pristane/phytane ratio	0.33	0.33

*Estimated concentration

TABLE IV-2. Aromatic Fraction of Santa Barbara Fresh and Weathered Crude Oil

Compound	Concentration (mg/g) Fresh Crude Oil	Weathered Crude Oil
Toluene	2.64	--
Ethylbenzene	0.38	--
p-Xylene	0.83	--
o,m-Xylene	0.39	--
Cumene	0.07	--
Mesitylene	0.15	--
p- Cymene	0.33	--
n-Propylbenzene	0.14	--
n-Butylbenzene	0.06	--
n-Hexylbenzene	--	--
Ethylmethylbenzene (2)	0.71	--
Methylpropylbenzene (3)	0.37	--
Dimethylbenzene	--	<0.010
Ethyldimethylbenzene (3)	0.46	--
Trimethylbenzene (2)	0.59	--
Dimethylpropylbenzene	0.13	--
Tetramethylbenzene (2)	0.12	--
Naphthalene	0.22	--
2-Methylnaphthalene	0.61	0.043
1-Methylnaphthalene	0.35	0.034
2-Ethylnaphthalene	--	0.039
Dimethylnaphthalene (4)	0.58	0.276
Trimethylnaphthalene (7)	0.47	0.363
Tetramethylnaphtalene	--	0.021
Ethyltetrahydronaphthalene	0.05	--
Trimethyltetrahydronaphthalene (2)	0.27	--
Benzothiophene	0.08	--
Dimethylbenzo/B/thiophene (2)	0.12	--
Ethylbenzo/B/thiophene	--	0.040
Methylfuorene	--	0.034
Dibenzothiophene	0.08	0.076
Methyldibenzothiophene (2)	--	0.087
Dimethylnaphtho/2, 3-B/thiophene (2)	0.07	--
Phenanthrene	--	0.032
Methylphenanthrene (3)	0.08	0.099
Dimethylphenanthrene	0.08	0.121
Dehydrodimethyl -1H-indene	0.09	--
Methyloxybiphenyl	--	--
Methoxydimethylbenzofuran	0.11	--
Total resolved hydrocarbons	24.98	4.01
Total unresolved hydrocarbons	120.60	22.10
Total hydrocarbons	145.58	26.11
Resolved/unresolved hydrocarbon ratio	0.21	0.18

TABLE IV-3. Polar Fraction of Santa Barbara Fresh and Weathered Crude Oil

Compound	Concentration (mg/g) Fresh Crude Oil	Weathered Crude Oil
Dimethylpheno l	*	--
Trimethylpheno 1	*	--
Hexadecanoicacid	*	--
Substituted benzenetricarboxylic acid	*	--
nC-11	*	--
nC-25	*	--
nC-28	*	--
Heptadecanol	*	--
Total resolved hydrocarbons	4.93	0.58
Total unresolved hydrocarbons	16.06	--
Total hydrocarbons	20.99	0.58
Resolved/unresolved hydrocarbon ration	0.31	--

*Concentration <().(),5 mg/g

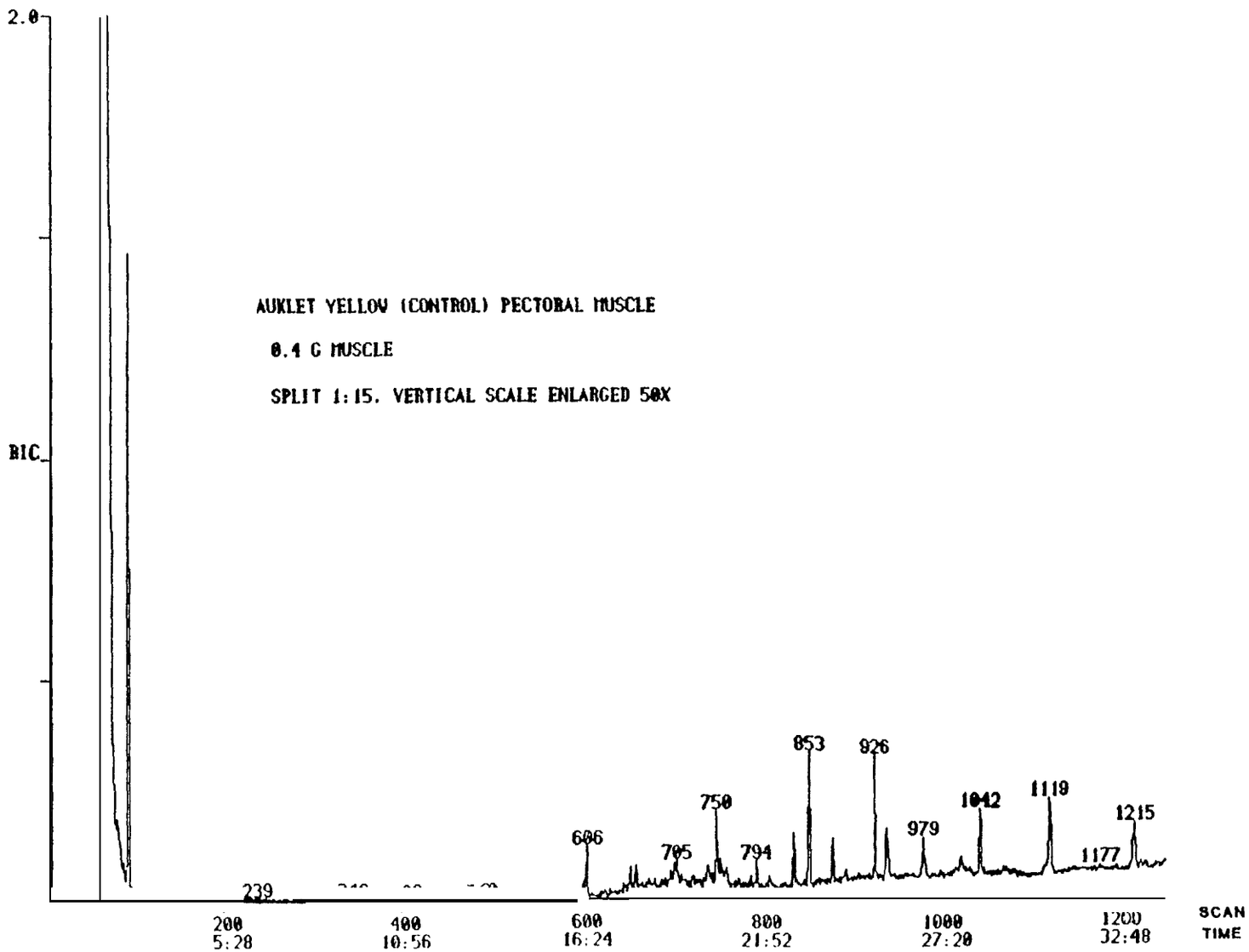


Figure IV-1. Reconstructed ion chromatograph of aliphatic hydrocarbon residues from the pectoral muscle of a captive control Cassin's Auklet. The major peak at Scan 853 is p,p'-DDE.

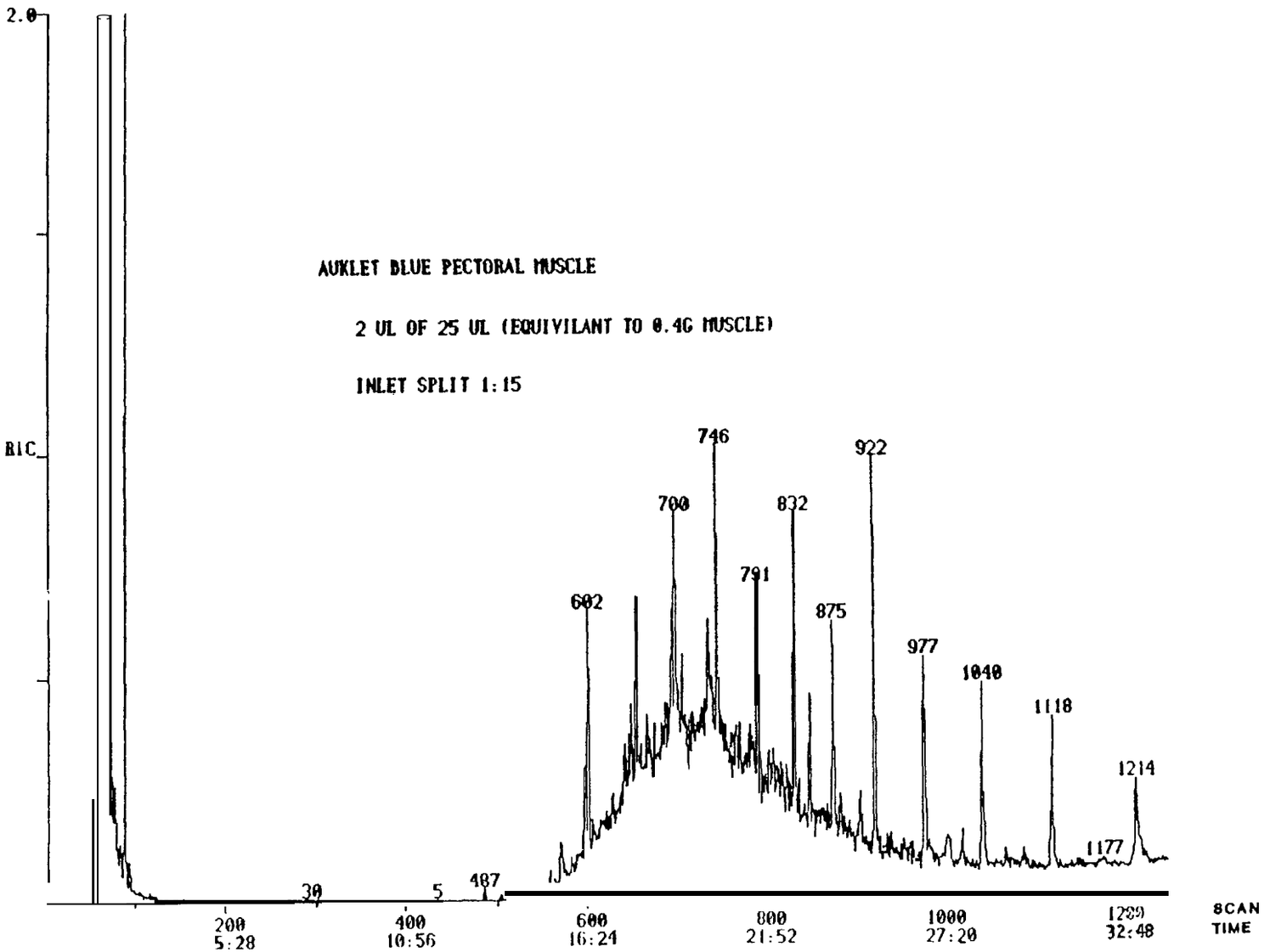
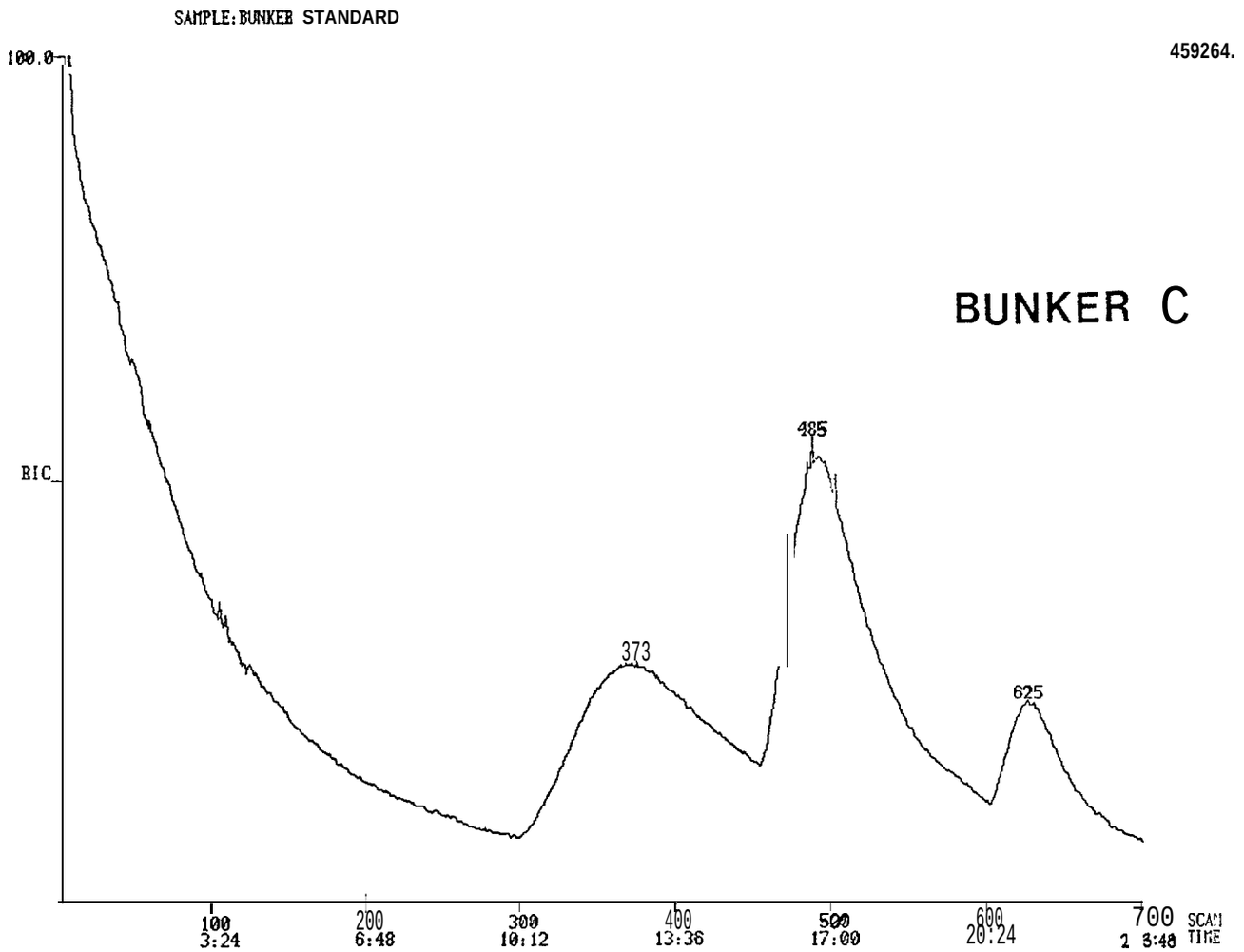


Figure IV-2. Reconstructed ion chromatograph of aliphatic hydrocarbon residues from the pectoral muscle of a captive Cassin's Auklet exposed to 3 ml of weathered oil four days previous to necropsy.



'Figure IV-3. Reconstructed ion chromatogram of the high vacuum distillation profile of Bunker C (No. 6 Fuel Oil) obtained from the Tanker "Puerto Rican".

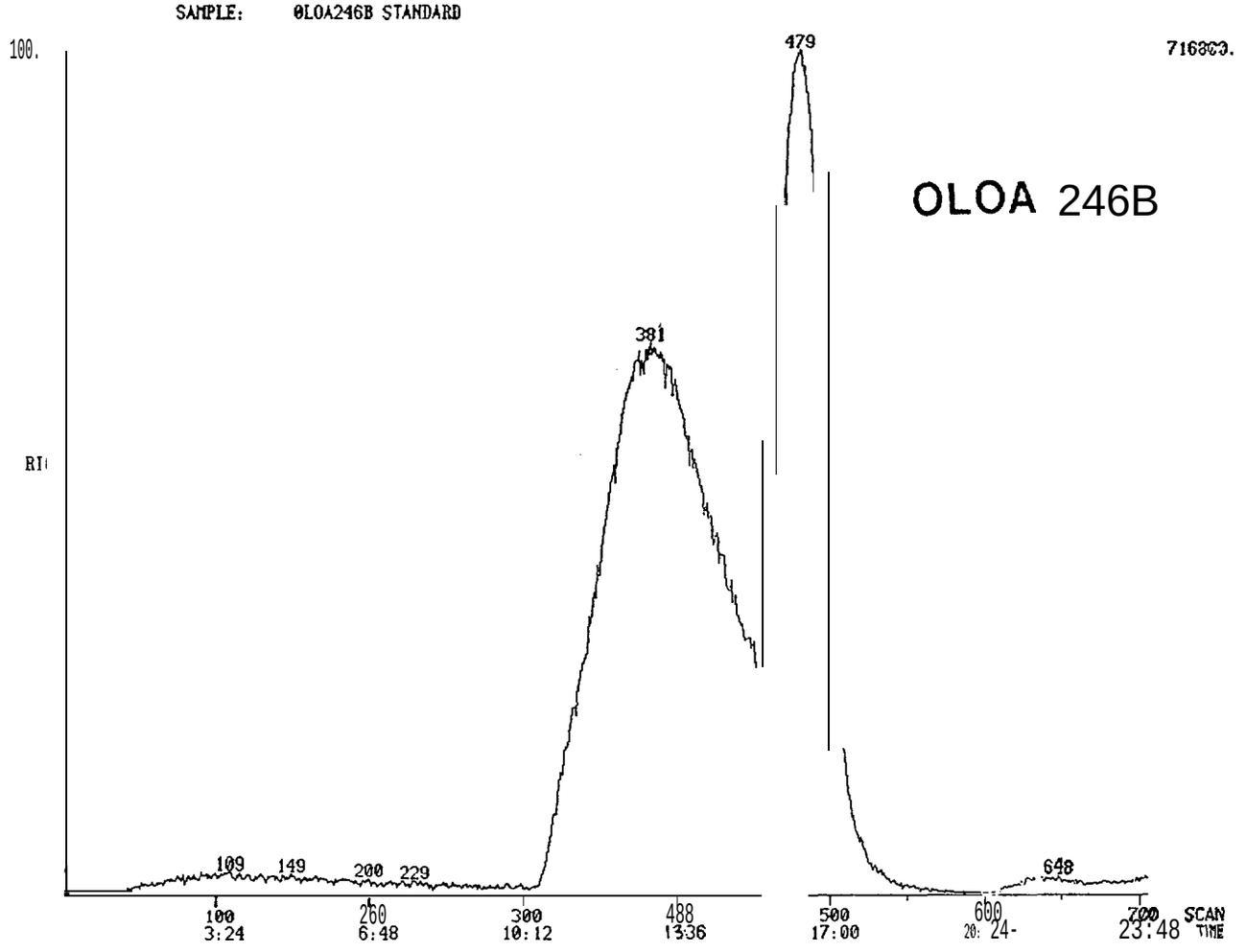


Figure IV-4. Reconstructed ion chromatogram of the high vacuum distillation profile of OLOA-246B obtained from the Tanker "Puerto Rican".

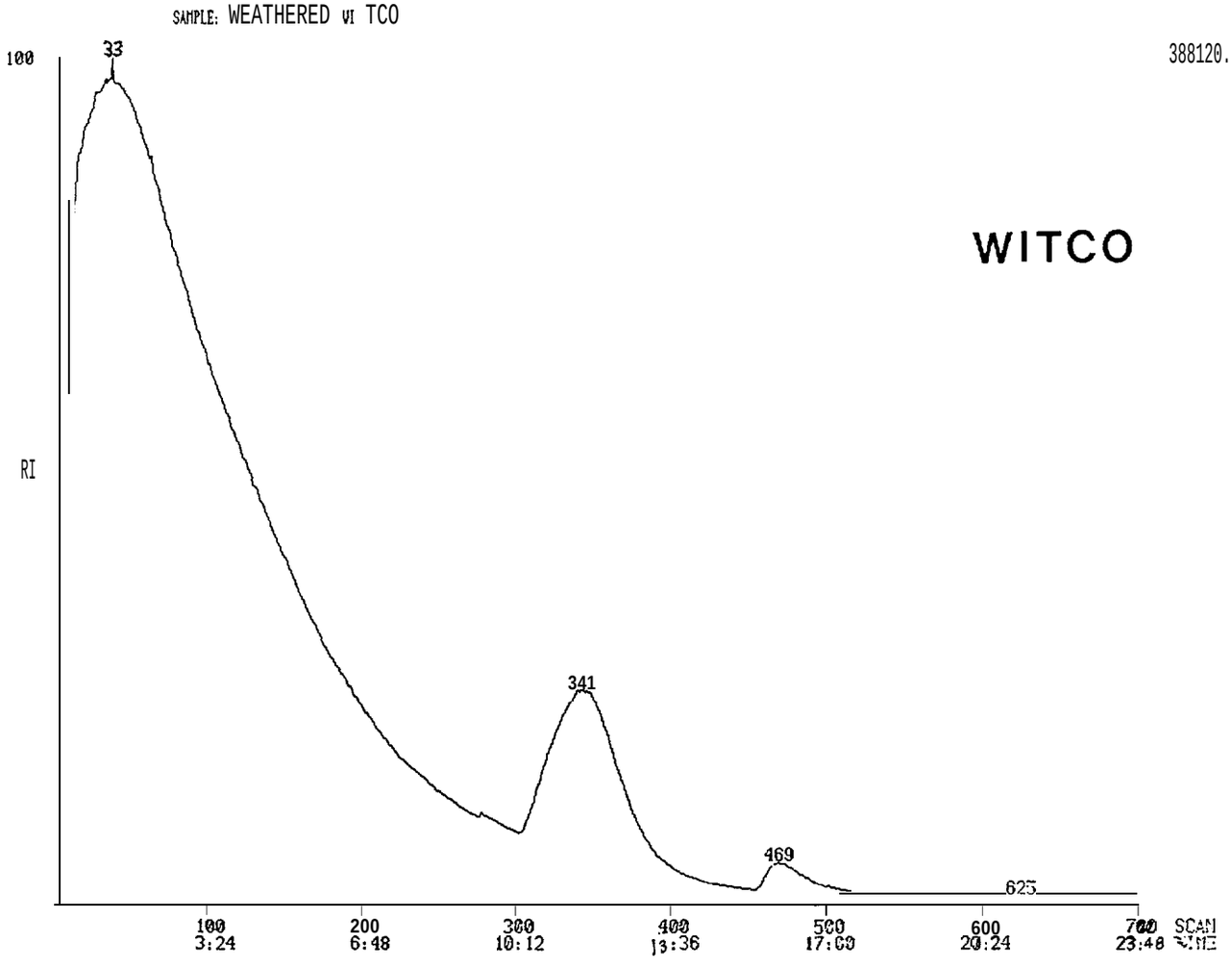


Figure IV-5. Reconstructed ion chromatogram of the high vacuum distillation profile of WITCO 2033TR lubricating oil obtained from the Tanker “Puerto Rican”.

contaminated with Bunker C fuel oil or mixtures of Bunker C and OLOA-246B (Figures IV-6, IV-7). In addition a few birds were contaminated with oil containing the dispersant Corexit 9527. The total amount of Corexit 9527 on oiled feathers appeared to be very low.

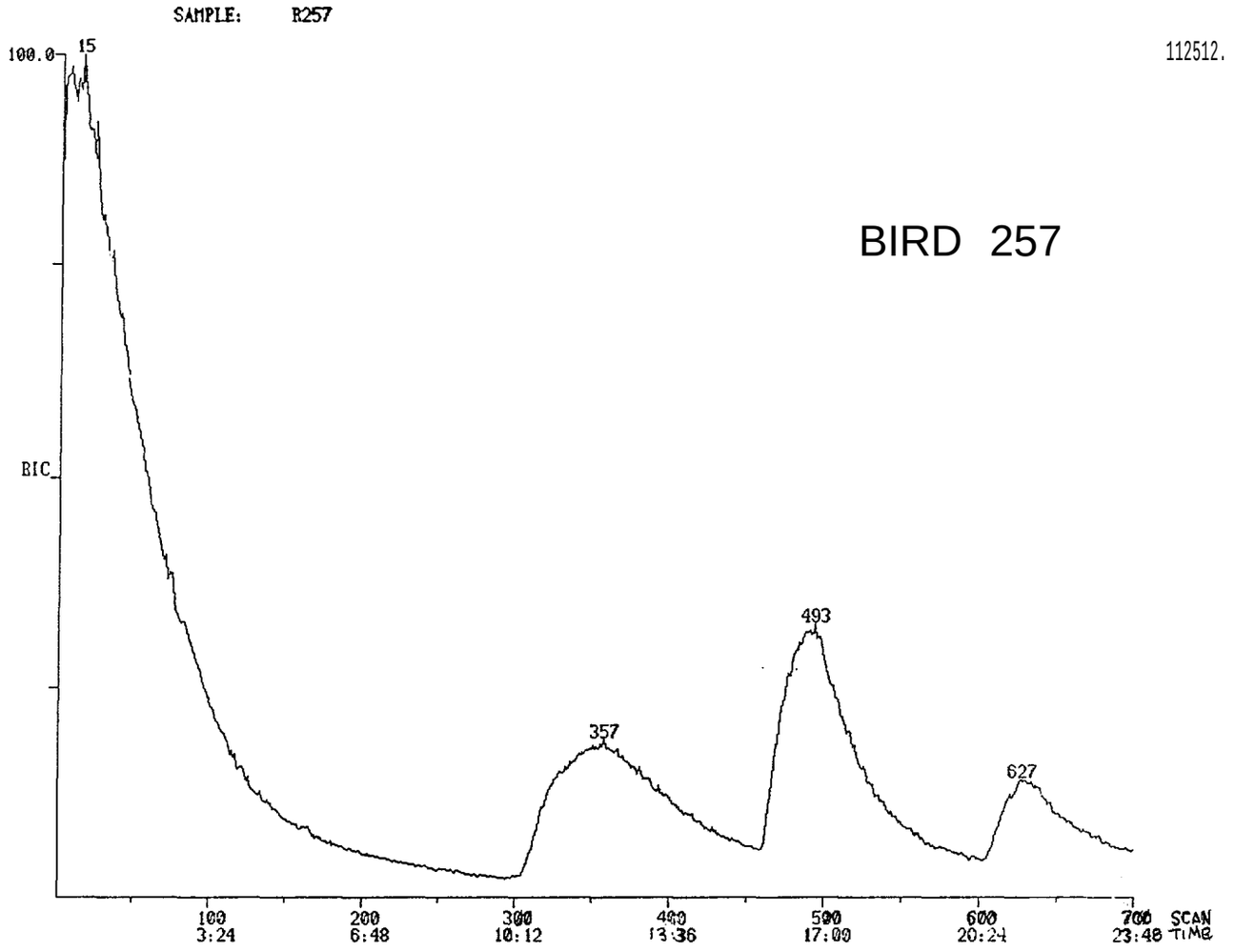


Figure IV-6. Reconstructed ion chromatogram of the high vacuum distillation profile of oil extracted from the feathers of a Common Murre oiled in the spill from the Tanker "Puerto Rican". This bird was oiled with Bunker C.

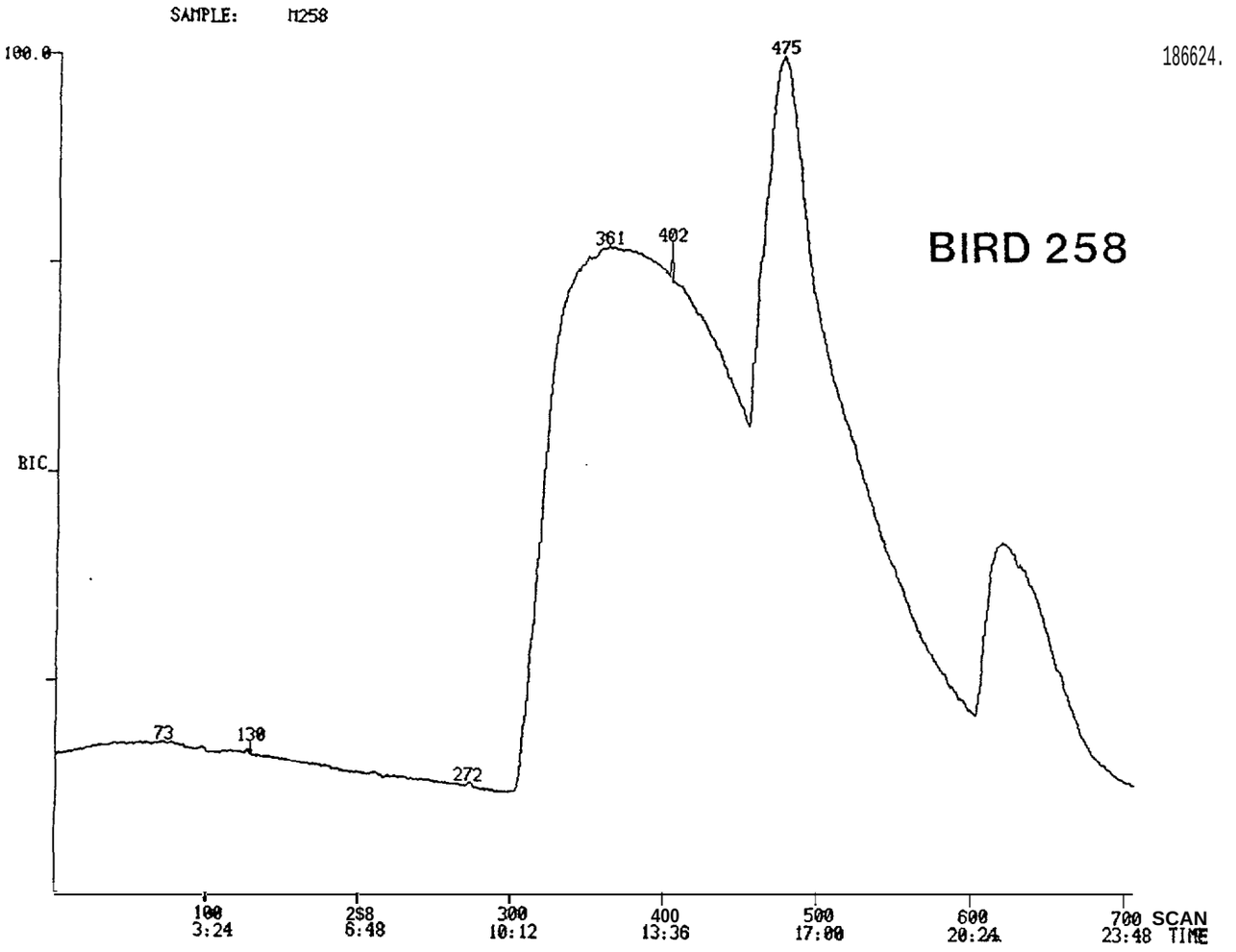


Figure IV-7. Reconstructed ion chromatogram of the high vacuum distillation profile of oil extracted from the feathers of a Common Murre oiled in the spill from the Tanker "Puerto Rican". This bird was oiled with a mixture of Bunker C and OLOA 246B.

V. PRELIMINARY EXPERIMENTS TO DETERMINE OIL DOSES TO BE USED FOR THE FIELD STUDIES

Preliminary studies were carried out at University of California, Davis (UCD) on domestic ducks and Cassin's Auklets to select a dosing regime that would deliver the maximum possible oil dose without so damaging the plumage that birds were at risk of death from exposure.

A. Extent of Weathering to Prevent Loss of Waterproofing of Birds

Small batches of crude oil were weathered for 1-6 days to generate products of increased viscosity and reduced solvent properties in order to prevent penetration of feathers and resulting waterlogging. Fresh crude oil was very liquid and soaked quickly with feathers of exposed birds. Loss of volatile solvents during weathering for more than 3 days resulted in a viscous and tarry product which remained on the surface of feathers. Trial batches of weathered oil were applied to domestic ducks to determine the extent of feather penetration, loss of waterproofing, and loss of buoyancy. White domestic ducks were subjected to external application of 2-5 ml of oil spread on the breast plumage. Two ml of fresh crude or oil weathered for one day caused the plumage of ducks to rapidly become waterlogged. When placed in a tank of water, the ducks rapidly lost buoyancy and immediately attempted to leave the water to begin preening. Examination of the breast plumage revealed matted plumage and water penetration into the underdown.

Crude oil, weathered for 2 or 3 days, similarly penetrated the plumage of domestic ducks, although to a lesser extent than oiled weathered for one day. Weathering for six days resulted in a much more viscous product which coated the feathers but remained on the birds' outer surface, even though it formed a nearly continuous layer on the surface of the breast. A duck oiled with 5 ml 6-day weathered crude did not lose waterproofing or float lower in the water than control ducks, and it was able to leave the water, preen, and re-enter the water many times without having the oiled plumage become waterlogged.

All oils applied to the breast plumage elicited an immediate and continued preening response which resulted in considerable oil ingestion by dosed ducks. Examination of the gut contents of these oiled birds revealed significant amounts of black residue with a distinct oil odor within the small bowel and cecae.

The experiments with domestic ducks demonstrated the necessity for using weathered crude oil with a viscosity similar to the six-day weathered product for external application to auklets and shearwaters in the field. If birds in the wild were exposed to a less viscous oil which would penetrate their feathers, they would quickly lose thermo-regulating ability and would be unable to enter the water to forage.

B. Preliminary Experiments to Determine Oil Doses for Cassin's Auklets

The acute captive experiment performed with Cassin's Auklets was helpful in determining the dosage levels of oil and the most suitable areas of the plumage for oil application. Three or 5 ml of weathered crude was applied either *to* the upper surface of the wings (secondary covert feathers) or to the central portion of the breast plumage. The upper surface of the wings was selected as an area that might minimize inadvertent contamination of eggs if birds remained oiled during incubation. Oil applied to the wings

was quickly preened onto the-primary and secondary feathers (see Figure III-2). Oil on the dorsal surface of the wings was therefore determined to be unacceptable, as flightless birds probably would not survive in the wild.

Exposure of 3 or 5 ml of oil to the central breast plumage proved too great a dose, even though auklets remained buoyant and their wings did not become oiled unless a number of treated birds huddled together. Three or 5 ml resulted in the death of captive auklets within 4 days.

The captive stress of auklets and suboptimal captive conditions precluded further trials to determine appropriate oil doses to apply to auklets. A subjective assessment of the amount of oil which could be tolerated by an auklet was made, and 1 ml was selected as a dose for preliminary field experiments.

c. Preliminary Dosing of Auklets in the Field

Because the optimum dose of weathered oil to apply to the plumage of auklets could not be determined in captive birds, an on-site study was designed and carried out on the Farallones, before the laying season began. From the results, the tendency to abandon the nest box could be assessed. This experiment on the external application of 1 ml of weathered crude to the breast plumage of auklets was carried out March 8-10, 1982. One ml covered nearly the entire breast of a Cassin's Auklet with a thin, viscous patch of oil.

All oiled birds and their prospective mates abandoned the box in which they were oiled. Half of the birds were subsequently found in different boxes on nightly banding checks. Smudges of oil were present on the plumage as long as two weeks after dosing, but the birds appeared healthy and did not lose weight as a consequence of the 1 ml dose. On the basis of these results, one ml was selected as the dose to be applied externally to Cassin's Auklets.

VI. FIELD STUDIES TO DETERMINE THE LONG-TERM EFFECTS OF INGESTED CRUDE OIL ON CASSIN'S AUKLET REPRODUCTION

A. Experimental Desire

The first species scheduled for oil dosing during the 1982 breeding season was Cassin's Auklet, because much background information on biology, breeding habits, and the acute response to oil was available (Grau, et al. 1977, Engel, et al. 1978, Ainley, et al. 1981). The original protocol called for four treatment groups of up to 75 pairs each. Both members of each pair were treated equally. The planned groups were: (1) Sham-dosed control; (2) Oral dose of 1 ml fresh crude; (3) Oral dose of 1 ml weathered crude; and, (4) external application of 1 ml weathered crude oil. Treatment group sizes were to be large, as the study was to be carried out over three years. If fewer birds were available in the first year of the study, the number of treatment groups was to be reduced and additional treatment groups would be included in the second year. Unfortunately, the second year a severe El Nino occurred (Schreiber and Schreiber, 1983; Donguy et al. 1983) and the planned dosing could not be continued in 1983.

Nest boxes were installed in two areas on Southeast Farallon Island (SEFI). One hundred forty-nine boxes were installed on the south slope of Lighthouse Hill immediately behind the PRBO house in an area likely to be the nesting area of birds caught in the fixed standing net used by PRBO for banding auklets (Figure VI-1: Areas Al and A2). An additional 345 boxes were installed on the Marine Terrace on the southeastern side of the island adjacent to the Carpentry Shop (areas B and C). A few of these boxes were destroyed early in the season by elephant seals. Installation of 494 boxes on the Farallones was completed on February 18, 1982. The boxes used for this study are referred to as "MMS" boxes; the boxes installed prior to this study are referred to as "PRBO" boxes or as "NOAA" boxes which had been installed in 1977 for a National Oceanic and Atmospheric Administration study.

The weather and breeding conditions on SEFI were highly variable during the course of this Study, and required extensive modification of the original experimental plan to develop successful experimental field protocols. Thus, there were actually three separate field studies conducted in overlapping years. In 1982, 65 adult auklets were exposed to oil prior to egg laying. The breeding success of these birds and a control group was determined in 1982, 1983, 1984, and 1985. No birds were dosed in 1983 when few birds bred due to the severe El Nine. A second study was begun in 1984 with an additional group of 35 auklets dosed externally and a group of 16 auklets dosed orally with 1 ml of weathered crude oil using a protocol similar to the 1982 study. The breeding success of these birds was determined during 1984 and 1985.

The third study was initiated in 1984 and consisted of external application of 1 ml weathered crude oil to auklets on day 14 or 15 of incubation, rather than prior to laying. The breeding success of these birds was determined during 1984 and 1985.

In all auklet studies, the following aspects of reproduction were monitored: identity of mate; date of lay; incubation attentivenesss; hatching success; chick growth; chick fledging date; and chick fledging weight. The same parameters were recorded if pairs relaid a second egg.

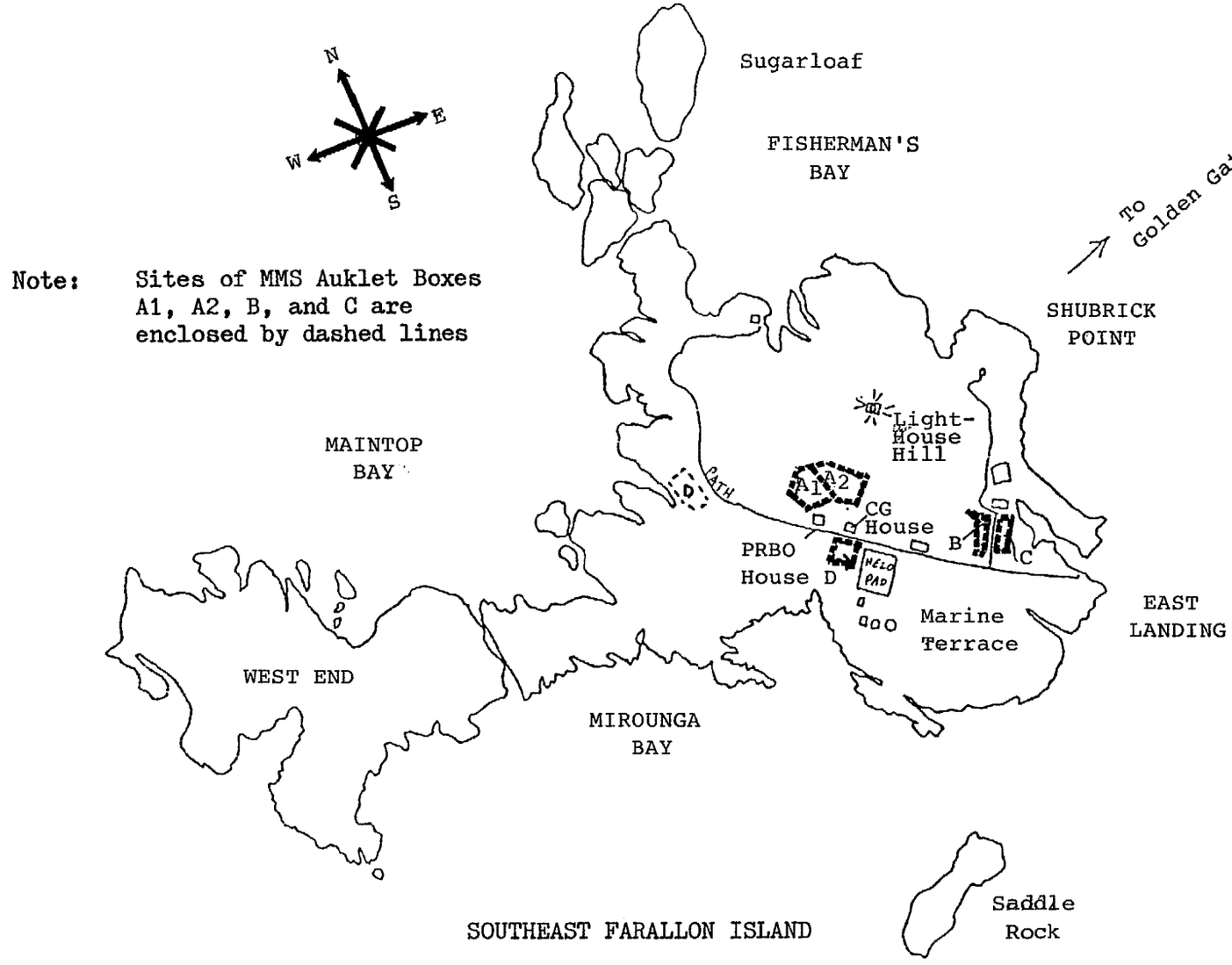


Figure VI-1. Map of Southeast Farallon Island, CA. Sites of MMS auklet boxes are enclosed within areas A1, A2, B, and C. NOAA auklet boxes are enclosed within area D.

Additionally, a study of hormone cycles in breeding auklets was attempted through measurement of excretory hormones and the urine and feces of auklets. Excretory hormones are routinely used in reproduction study of mammals, and this study afforded a unique opportunity to monitor hormones in free-living birds. Fresh croppings were routinely collected from all auklets when they were handled. Auklets frequently defecated when held, and samples could easily be collected in small plastic bags (NASCO whirl-pats) without increasing disturbance to the birds. Each sample was labeled with the date, time, and band number of the bird. The processing methods and hormone measurements obtained in this study are included as an Appendix to this report.

Each of the studies is presented in narrative form to explain the "real-time" modifications of the protocol. Summaries of each year's results are also given. The analysis of the effects of oil have been treated separately and comparisons have been made where possible.

B. Auklet Study I: External Application of Weathered Crude Oil Prior to Lay in 1982

The seabird breeding season on the SEFI correlates with the seasonal occurrence of oceanic upwelling off central California. Upwelling of nutrient-rich subsurface water stimulates phytoplankton growth which supports large populations of euphasiids, small crustacea which are the principal food resource of spawning rockfishes and many seabirds. The alcids and cormorants breeding on the Farallon Islands feed on crustacea and juvenile rockfish and are therefore dependent upon upwelling for successful breeding (De Sante and Ainley 1980).

1. 1982 Field Season

a. Weather and Breeding Chronology

Auklets arrive and depart the island only in darkness to escape predation by gulls. Courting auklets normally dig burrows or clean out old burrows early in the season and may be found in the burrow (or nest box) during many nights for a few weeks prior to laying. Before laying, no adults remain in burrows during the day. When the egg is laid, one bird incubates all day and its mate returns during the night for the nest relief. During incubation both birds can usually be found in a nest chamber at night. Incubation shifts are usually one day, making it convenient to read bands of both adults or collect droppings by visiting a nest during the day on successive days. When disturbed during the day, auklets will not abandon the nest but will remain in the box until dark.

January and February 1982 were slightly colder than normal, which indicated an early beginning of the breeding season. This initial false start to the breeding season was followed by warm weather and light winds resulting in reduced upwelling in March and April and correspondingly high surface water temperatures.

On SEFI, auklets normally begin to lay in March with the majority of birds usually laying within a three- to four-week period. The first eggs laid in MMS boxes were on March 8 and March 10, 1982, but were incubated infrequently or not at all and were discarded soon after laying. No further eggs were laid until April 5, when one of the control-group pairs that had laid on March 10 relaid in a different box. That egg was never incubated (the female switched mates and laid a third egg May 4).

The unusual weather continued throughout April and into early May with high ocean temperatures. During the first week of May, 16 pairs were incubating and an additional 22 laid eggs. Of the 16 pairs incubating, 11 abandoned eggs during the first week of May. In other boxes, 50% of the eggs laid during that week were never incubated. None of the 18 birds dosed with oil on May 5 or 7 were seen again in 1982.

Increasing winds began on May 9 causing the resumption of upwelling, and the ocean temperature cooled to 10° C for the rest of the month. Thirty-nine more pairs laid during late May including 8 pairs which relaid after abandoning an earlier egg. Those pairs which incubated regularly in early May managed to hatch chicks in June although many pairs abandoned during mid-June when the weather again turned warm and euphausiid populations dropped.

The auklet laying period in 1982 was protracted (Figure VI-2). Cormorants and murres also began laying later than usual. Many auklets abandoned their eggs laid in March or April, and relaid in May when ocean temperatures dropped. Good breeding conditions prevailed into June at which time water temperatures again rose 5 C above normal and the prey base for the breeding birds failed. Pigeon Guillemots and Pelagic Cormorants abandoned eggs and many chicks died. Most auklets continued to feed their chicks throughout the early summer, but with the decreased food availability, chicks were forced to fledge at 55-80 percent of normal weight.

A few auklets continued to attempt to raise chicks and two pairs even laid eggs after June 27. One of the late pair hatched their egg and fledged a chick in the last week of August. All of the chicks were banded during July and August before fledging.

b. Oil Application and Results

Banding commenced on March 8 but few birds were found, as occupation of nest boxes was less than 15% on any night early in March and April. Many banded birds were found in different boxes on subsequent nights (the first bird banded, #10701, was captured 9 times in 8 different boxes between March 8 and May 21, when its mate laid in a box 15 feet from the box in which it was banded). Three hundred fifty-eight auklets were banded in March. Twenty additional auklets previously banded by PRBO were found occupying boxes during March. Fewer than 60' pairs of these banded birds were found again in March, although some were discovered in boxes later in the season.

The extent of box switching made it impossible to predict where auklets would be found until an egg was laid, which committed the pair to that nest box. As a result every unoccupied box was checked each day for eggs or incubating birds from March through June.

In the original protocol, the plan was to dose pairs beginning the night after the first eggs were laid. But, because the first three eggs were early compared to the population as a whole, it was decided to wait until birds in established PRBO boxes began laying before dosing birds for the main study. Dosing was begun

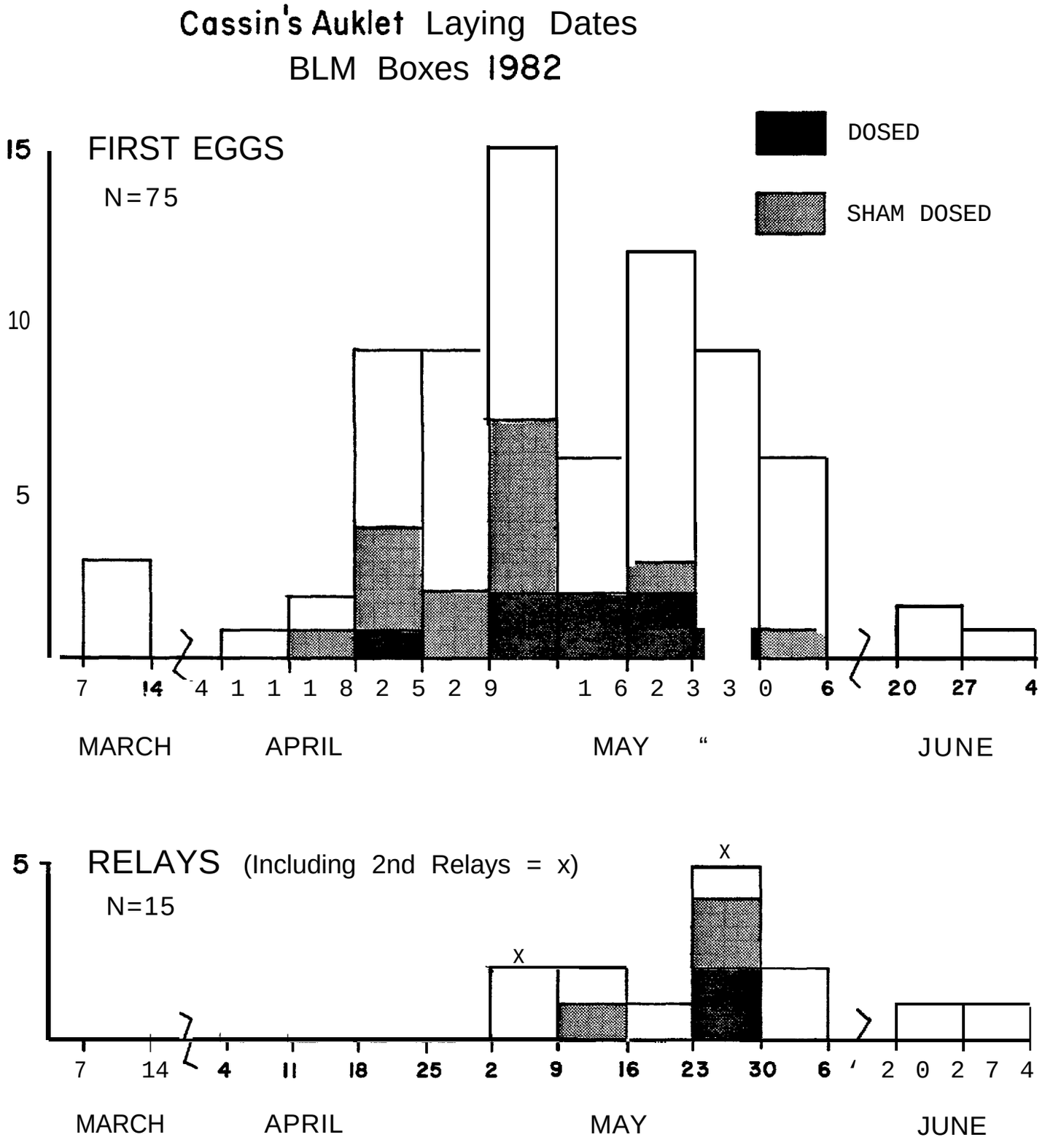


Figure VI-2. Laying dates of control and experimental Cassin *s Auklets, 1982.

on April 9 based upon the breeding activity of established breeding pairs monitored by PRBO.

The unexpected box-switching behavior of banded birds forced a protocol change in which only pairs of birds that had been observed together on a previous occasion were dosed. Dosing was attempted on alternate nights, but because of low occupancy only 24 birds were dosed during the period of April 9-16.

Small numbers of birds were dosed through late April with a total number of 49 birds being dosed (one bird escaped as its mate was being dosed) and 38 birds handled as "sham-dosed" controls. Dosing was extended into early May on two nights (May 5 and 7) with 18 additional birds dosed, bringing the total to 65. All 65 birds were dosed externally with 1 ml weathered crude oil applied to the breast feathers.

Throughout the breeding season, inspection of nest boxes was performed daily to record egg laying and to collect droppings for hormone analysis from incubating birds on the schedule outlined in the original protocol. Incubating birds were checked on Day 1 when the egg was first found, the mate was checked on Day 2, and birds were thereafter handled only on Days 4, 5, 14, 15, 24, 25, 34, 35, and after the chick hatched. Handling of incubating birds for collection of fecal samples continued through the early chick period. Droppings were obtained from birds by holding them for a short period of time (one minute or less) over the mouth of a small "whirl-pat" plastic sampling bag and labeling the bag with the date and band number of the bird sampled. Although droppings were not obtained every time a bird was handled, this appeared *to* be the quickest and least stressful method of collection.

In summary, 488 banded birds occupied MMS boxes during 1982. Four hundred and eight were banded in boxes and 80 had been previously banded, either as chicks or after being caught in the PRBO banding net. Sixty-five adults were dosed, 10 dosed pairs laid and 2 fledged chicks. 37 auklets were handled as "sham-dosed" controls, 13 pairs laid and 3 fledged chicks. Fifty-seven other non-experimental pairs were banded when they laid in MMS boxes and these fledged 12 chicks (Table VI-l).

Dosing birds with oil resulted in many abandonments. The most striking difference between dosed and control groups was abandonment before laying by the dosed birds; 43 of the 65 oil exposed birds (66%) were not seen again in 1982 while only 5 of 38 (13%) of the sham-dosed birds disappeared. Dosing birds in late April and early May when the weather was unfavorable for breeding caused much abandonment. 26 of 36 birds dosed after April 19 abandoned without laying. It is likely that most birds occupying boxes late in the year had a low commitment to breed in 1982 or were sub-adult birds occupying nests for the first time. However, only 5 of 18 controls banded, measured, and "sham-dosed" during the same period abandoned without being seen again in 1982.

The breeding success of those dosed and control birds which did not abandon their nests was similar (Table VI-l). Both groups had equal hatching success and equal fledging success. Several dosed

TABLE VI-1. 1982 BREEDING SUCCESS OF CASSIN'S AUKLETS*.

	CONTROL	EXTERNAL DOSE
NUMBER	38	65
NO. NOT SEEN AGAIN IN 1982	5	43
NO. OF BIRDS REMAINING AFTER DOSING	33	22
% OF BIRDS REMAINING AFTER DOSING	86.5%	33.8%[a]
NO. OF BIRDS LAYING EGGS	25[1]	17[2]
% LAYING EGGS	66%	26%[b]
DOSE TO LAY INTERVAL (DAYS)	17.1 ± 11.93	41.8 ± 18.2[4,c]
NO. OF BIRDS HATCHING EGGS	10	6
HATCHING SUCCESS (%)	38. 5%	30. 0%
NO. OF BIRDS FLEDGING CHICKS	6	4
FLEDGING SUCCESS (%)	60. 0%	66.7%

*: Chi-square analysis with Yate's correction.

a: Significantly fewer external dose birds remained in the colony than control birds after dosing ($p<0.05$).

b: Significantly fewer external dose birds laid eggs than control birds after dosing ($p<0.01$).

c: A significantly longer dose to lay interval was experienced by external dose birds than control birds ($p<.001$).

1: Includes one pair that laid an egg on the day of sham-dosing.

2: Includes one pair that laid an egg 1 day after dosing.

3: Excludes one pair that laid an egg on the day of sham-dosing.

4: Excludes one pair that laid an egg 1 day after dosing.

birds that were not seen after dosing in 1982 returned in 1983 or 1984. We believe the disappearance of most or all dosed birds to have been desertion rather than death because birds observed after dosing did not appear weakened, emaciated, or fouled in any way.

Oil exposure caused a significant delay in egg laying after dosing. Only one bird (which had an egg in-the shell gland when dosed) laid an egg sooner than 20 days after oil exposure, and the delay in others indicated probable disruption of egg formation and a delay in initiation of new follicles. Ten of 38 controls which were sham-dosed during egg formation laid eggs 5-9 days after sham-dosing. None of the 65 dosed birds laid during this interval after dosing. The average interval between sham-dosing and laying of controls was 17.1 days in contrast to an interval of 41.8 days between dosing and laying of oil dosed birds.

A previous study (Ainley et al. 1981) indicated that force feeding auklets 1 ml of Prudhoe Bay crude oil resulted in the delay or suppression of ovarian follicle formation which would have been initiated at the time of dosing. The usual time to re-lay after loss or removal of a Cassin's Auklet egg is 15.8 $\pm$ 1.2 days (Astheimer 1984). The minimum time to lay after dosing was 20 days and the single dosed bird which laid and abandoned its egg the day after dosing relaid a second egg 20 days later. The additional 4 day lag in laying or re-laying is similar to the interruption found by Ainley et al (1981) after oral dosing with Prudhoe Bay crude or Bunker C oils.

2. 1983 Field Season

The first follow-up year for birds dosed in 1982 proved to be an extremely atypical year for breeding seabirds on SEFI. A very strong El Nino-Southern Oscillation caused a warming trend in the eastern Pacific Ocean which progressed up the Coast of North America and caused failure of breeding seabirds throughout California, Oregon and elsewhere.

On SEFI, the breeding populations of most seabird species were extremely reduced in 1983, with Brandt's Cormorants, Pelagic Cormorants, and Tufted Puffins all experiencing nearly complete breeding failure. Western Gulls laid eggs, but hatching success was poor and chicks were primarily fed garbage because of the failure of the normal food supply (juvenile rockfish). Adult gulls were forced *to* fly to the mainland and forage in landfills for food. Common Murres responded to a period of good weather in May and approximately 45% of nest sites monitored by PRBO had eggs in June. Nearly all of the eggs, however, were abandoned because of the failure of the rockfish spawn.

The MMS artificial nest-boxes had very low occupancy in 1983 and by the end of May only 4 boxes had eggs. In total, 77 Cassin's Auklets were handled during 1983. Forty of the birds had been banded in 1982 and 37 were newly banded in 1983. Of the 40 banded birds, 9 were controls, 5 were oiled during the 1982 Study, and 26 were non-treatment controls. A total of 6 eggs were laid in nest-boxes on the Farallons during 1983, one egg laid by a pair of externally dosed auklets and 5 eggs by birds newly bandied in 1983. No 1982 controls or non-experimental birds laid eggs. All eggs were abandoned and no pairs relaid, resulting in 100% breeding failure of all auklets monitored in 1983 (Table VI-2).

TABLE VI-2 RETURN OF CASSIN'S AUKLETS IN 1983 AND 1984.

1982 CONTROL BIRDS

	not seen again	seen 83 only	seen 84 only	seen 83 and 84
Birds not laying in 1982(X)	58	8	25[a,d]	8
Birds which laid in 1982(%)	40	36[e]	4	20

1982 EXTERNALLY DOSED BIRDS

	not seen again	seen 83 only	seen 84 only	seen 83 and 84
Birds not laying in 1982(%)	78	16[b]	4	2
Birds which laid in 1982(%)	56	0	13	31[c]

a. Significantly greater return than 82 Control Layers in 1984 ($p<0.05$).
b. Significantly greater return than 82 Dosed Layers in 1983 ($p<0.05$).
c: Significantly greater return than 82 Dosed Non-layers in 1983 and 1984 ($p<0.001$).
': Significantly greater return than 82 Dosed Non-layers in 1984 ($p<0.05$).
': Significantly greater return than 82 Dosed Layers in 1983 ($p<0.01$).

Dosing was to have been continued in 1983 to increase the sample size of oiled birds, but was cancelled due to low occupation of boxes. The total number of birds returning to the island in 1983 was so low and the breeding conditions so unusual that no analysis of the effect of oil could be made.

3. 1984 Field Season

Early storms in January 1984 initiated upwelling and began to bring water temperatures down confirming the end of El Nine. Northwest winds in February brought expectations of an "average or better" breeding season for all species on the Farallones.

A large number of birds of all species bred on SEFI in 1984, an increase, perhaps, because of the poor year in 1983. Auklets began laying in late March. A high proportion of PRBO and NOAA boxes had eggs by April 1.

Only a small number of 1982 dosed and control auklets returned in 1984 as many birds apparently died due to El Nine. Breeding records of auklets monitored by PRBO indicated more than a 50% turnover in auklets occupying PRBO and NOAA boxes, and a large decrease in the breeding population of murres over the entire island. Ten 1982 controls and ten 1982 dosed auklets each were identified in MMS boxes, with seven of the controls and six dosed auklets laying eggs in 1984. Therefore, of our 103 total birds from the 1982 season, 29 had been located in 1983 and 20 returned to MMS boxes in 1984. The number of returning birds was low, and the influence of El Nino undoubtedly contributed to the poor return. Significant trends, however, were present in the data.

The data show that significantly fewer dosed birds returned compared to controls (Table VI-2). Additionally, a greater proportion of birds which laid eggs in 1982 after being handled or dosed returned in 1983 and 1984 compared to birds which did not lay in 1982. Sixty percent of controls which laid in 1982 returned in 1983 and/or 1984, while only 42% of birds which did not lay in 1982 returned. A similar trend occurred with dosed birds: 44% of 1982 layers returned, while only 22% of 1982 non-layers returned.

Breeding success in 1982 had a significant effect on the site faithfulness of birds returning in 1983 and 1984. Most of the control birds which returned to lay in MMS boxes in 1983 or 1984 were birds which successfully hatched an egg in a MMS box in 1982 (8 of 13 birds). Ten control birds which laid an eggs in 1982 did not return in 1983 or 1984, Nine of the 10 failed to hatch their egg in 1982, and may have moved to other parts of the colony to breed in subsequent years. The evidence for dosed birds is nearly identical: nine dosed birds which laid in 1982 did not return in 1983 or 1984; 8 of the 9 did not hatch their egg in 1982. In contrast to the failure to return of controls which did not hatch an egg, a positive trend is shown for the return of some dosed birds. Four of the 6 auklets which returned to lay in 1983 and 1984 were birds which laid but failed to hatch chicks in 1982. These birds may have failed because of the oil, but returned to *try* again. Only 3 of the 49 dosed birds which abandoned the breeding season before laying eggs in 1982 returned to lay in 1983 or 1984, and none of these birds hatched eggs. Since 66% of all dosed birds abandoned without laying in 1982, this represents a large and significant proportion of the oil-exposed population.

We conclude that disturbance caused by oil exposure resulted in breeding failure due to abandonment, and additionally, that auklets which failed to hatch an egg did not usually return to the same breeding site in subsequent years. Whether these birds bred in natural burrows is unknown; however, none of these birds bred in any of the PRBO or NOAA boxes in 1983, 1984, or 1985.

The return of experimental auklets banded in 1982 showed a definite sex bias with respect to their response to oil exposure (Table VI-3). The proportion of males resighted in the MMS boxes is independent of oil exposure. The return of females, however, is negatively correlated with oil exposure, with a low return of dosed birds.

The refighting figures were also calculated for all birds which laid eggs in 1982; they are given in Table VI-4. The trend is the same as for the treatment group as a whole, but the sex bias and dosing effect are more pronounced.

Oil exposure had no measurable effect on the return of male auklets to MMS boxes. A slightly larger percentage of males whose mates laid in 1982 returned than those which abandoned the season early, but there were no differences in return of control and dosed males. Oil exposure of females, however, had a significant effect on return in subsequent years. Seventy-seven percent of all control females which laid in 1982 returned in 1983 and/or 1984, while only 22% of dosed females returned. The proportion of females returning was independent of laying success in 1982 (Table VI-5).

The small sample size of returning auklets and the confounding introduction of El Nino makes it impossible to form conclusions as to the breeding behavior and site fidelity of auklets exposed to oil.

None of the auklets returning in 1984 were found with their mates of 1982, and only one bird (a control male) was found in the same box it occupied in 1982. Mate fidelity may have been independently disrupted by El Nine, but further studies would be useful to determine whether male and female auklets respond differently to interference with breeding.

4. *1985* Field Season

Nine auklets from the 1982 study returned in 1985: six controls and 3 dosed birds. The 1985 breeding success of these birds was very good: 5 of 6 controls and 3 of 3 returning dosed birds fledged chicks (Table VI-6). Most of the returning birds had bred in MMS boxes in 1984. Only 1 of the 3 dosed birds did not breed in 1984, and that bird had not been seen since it laid, but failed to hatch an egg, in 1982. Two of the six control birds had not returned in 1984; both had failed to hatch eggs in 1982 and one had not been seen since. The other returned in 1983 with its original mate, but did not lay. One auklet, which had abandoned in 1982 after being dosed, returned in 1985 and successfully fledged a chick. This was the only dosed bird that abandoned in 1982 and ever returned to breed and successfully fledge a chick. The breeding success in 1985 was very high for 1982 returning birds, perhaps because these birds were becoming experienced breeders.

Table VI-3: Sightings of all Cassin's Auklets dosed in 1982.*
Numbers of birds seen in 1983 and/or 1984.

	MALES		FEMALES		UNKNOWNS		TOTALS	
TREATMENT	#[1]	%	#	%	#	%	#	%
CONTROL	8/18	44.4[a]	12/18	66.7[b]	0/2	0.0	20/38	52.6
DOSED	9/30	30.0[a]	8/31	25.8[b]	0/4	0.0	17/65	26.2

*: Chi-square analysis with Yate's correction.

1: # = No. seen in 1983 and 1984 / No. treated in 1982.

a: Resightings of males was independent of dosing ($P>0.250$).

b: Resightings of females was negatively correlated with dosing ($P<0.05$).

Table VI-4. Sightings of Cassin's Auklets which were members of pairs that laid eggs in 1982.
Numbers of birds seen in 1983 and/or 1984.

	MALES		FEMALES		UNKNOWNS		TOTALS	
TREATMENT	#	%	#	%	#	%	#	%
CONTROL	5/12	42	10/13	77	0/0	-	15/25	60
DOSED	3/7	43	2/9	22	0/0	-	5/16	31

Table VI-5. 1984 Breeding Success of 1982 Experimental Auklets.

	Control	Dosed
No. Returning/Total	9/38	9/65
% Returning	24%	14X
No. of birds laying egg	7	7
% Laying eggs	78%	78%
No. of birds hatching eggs	4	2
Hatching success (%)	57%	29%
No. of birds fledging chicks	o	1
Fledging success (%)	o%	50%
No. of birds relaying eggs	3	3
No. of birds hatching 2nd egg	1	0
No. of birds fledging 2nd chick	0	0

Table VI-6. 1985 Breeding Success of 1982 Experimental Auklets*.

	Controls	Dosed
No. Returning/Total	6/37	3/65
% Returning	16%	5%
No. of birds laying eggs	6	3[a]
% Laying eggs	100%	100Z
No. of birds hatching eggs	5	3
Hatching success (%)	83%	100%
No. of birds fledging chicks	5	3
Fledging success (%)	100%	1 00%
No. of birds relaying eggs	1	0
No. of birds hatching 2nd egg	o	0
No. of birds fledging 2nd chick	o	0

*: Chi-square analysis with Yate's correction.

a. Significantly fewer number of eggs laid by dosed birds than control birds ($p<0.05$).

In summary, three of 65 (4.6%) birds dosed in 1982 and 6 of 37 (16.2%) controls returned *to MMS* boxes in 1985. The data remain consistent through all years, with a lower percentage of dosed birds returning, but the number of birds was so low in 1985 that no firm conclusions can be reached.

C. Auklet Study II: Oral and External Oil Treatments Prior to Laying in 1984

1. 1984 Field Season

Two separate oil-dosing experiments were carried out in 1984: exposure prior to laying; and exposure on day 14 or 15 of incubation. The protocol for pre-lay dosing was the same as in the 1982 study, with the addition of a treatment group of birds dosed orally with 1 ml weathered crude oil in a No. 00 gelatin capsule. Because a high proportion of the externally oiled group abandoned in 1982, the group size of this treatment was planned to be twice the size of the control and oral dose groups.

PRBO biologists checked the auklet boxes on the nights of March 10 and 19. One pair and ten single birds were occupying boxes on the 10th, indicating the season had only just begun. On March 19, 30% of the boxes checked (46 of 152) were occupied. Similar numbers of birds were found on the night of March 22, with more pairs than single birds present.

Dosing was begun on the night of March 25 when 25 pairs were dosed (17 external, 8 oral) and 8 pairs were handled and selected as controls. The attendance of birds at night dropped in late March. Undisturbed auklet boxes monitored every year by PRBO were checked to compare occupancy and determine whether night visits to MMS boxes were causing abandonment. Most of the PRBO boxes had breeding pairs with eggs, indicating that disturbance at night was having an adverse impact on the occupancy of MMS boxes. Night visits were curtailed for four nights to allow birds to return, but a reduced number of birds were found on March 31. Night box checks were stopped as were all banding and dosing at night. The final sample size of our treatment groups in 1984 was: control: 14 birds; oral dose: 16 birds; and external dose: 34 birds.

Four of the orally dosed birds and 3 of the externally dosed birds were recaptured at night 6 days after dosing for blood cell measurements and examination of blood smears for possible Heinz bodies. The results of these determinations are presented in Section III-D.

The small pre-lay dosing study of 1984 (Table VI-7) produced almost identical results to the study of 1982. A high proportion of the externally dosed birds abandoned the breeding season without laying eggs (70.6%) compared to 75.4% abandonment of the 1982 dosed birds. Control birds in both years remained in the area and most laid eggs (52.6% in 1982 and 57.1% in 1984). Hatching success of externally dosed birds in 1984 was lower than controls (50% vs. 62.5%) but not statistically different. Fledging success was similar in both groups (40% for externals, 44.4% for controls).

Sixteen adults were dosed orally with 1 ml of weathered Santa Barbara crude oil in 1984. The laying frequency (75%), hatching success (66.7%) and fledging success (75%) were all very high and showed no effect of the oral dose on breeding success.

Table VI-7. Fates of first eggs by experimental Cassin's Auklets treated before lay in 1984.

	CONTROL BEFORE LAY	ORAL-DOSED BEFORE LAY	EXTERNAL-DOSED BEFORE LAY
No. treated	14	16	34
No. seen after treatment	11	12	12
% seen after treatment	79%	75%	35%
No. of birds laying eggs	8	12	10
No. of birds laying eggs with treatment mate	6	10	4
% laying eggs with treatment mate	75%	83%	40%
Dose to Lay Interval* (Days)	24.0 ± 11.3	32.5 ± 12.5	44.8 ± 12.9[a,b]
No. of birds abandoning first egg	0	4	2
% abandoning first egg		33%	20%
No. of eggs hatched	5	8	5
Hatching success (%)	63%	75%	50%

*: Student's t-test.

a. Significantly longer dose to lay interval than control birds ($p<0.01$).

b. Significantly longer dose to lay interval than oral dose birds ($p<0.05$).

Oil dosing resulted in a delay in egg laying similar to the delay observed in 1982. Control auklets laid eggs an average of 24.0 days (range 16-36) after sham-dosing while orally dosed birds laid an average of 32.5 days (range 15-52) after dosing, and externally dosed birds laid an average of 44.8 days (range 23-74) after dosing (Table VI-7). All birds in this study were dosed or sham-dosed early in the season (March 25-27) and the slightly longer delays seen in 1984 compared to 1982 could be a result of working early in the season.

In summary, external dosing with weathered crude oil caused significantly greater abandonment resulting in significantly fewer eggs laid by dosed birds compared to controls. Oil exposure resulted in a delay in egg laying with external dosing causing a greater interruption than an oral dose. Those dosed birds which did lay, however, hatched and raised chicks as successfully as controls. Oral dosing with 1 ml weathered crude oil had no effect on laying frequency or breeding success in the year of dosing.

2. 1985 Field Season

There was no difference in the percentage of control or oil exposed auklets that returned in 1985 (Table VI-8). The return of males and females, however, was skewed, with more males returning to breed than females. The small number of birds returning precluded the conclusion that dosing or handling per se caused a low return to the MMS boxes in 1985, but the pattern of females abandoning in the year following disturbance is the same pattern as in the 1982 dosing experiment. Many auklets appeared committed to the breeding season in the year of oil exposure and were tenacious in remaining to breed, but females appeared to leave the area in the subsequent year while a greater proportion of the males remained in the area to breed. It is not known whether the females bred successfully elsewhere.

The results shown here could be a manifestation of philopatry and territoriality in Cassin's Auklets similar to several other species of seabirds and gulls (Greenwood 1980; Chabrzyk and Coulson 1976; Fox and Boersma 1983). If males return to their natal site for breeding and females tend to choose a site away from their natal site, then females may have a much greater tendency to move away from an area of disturbance.

The overall effect of oil exposure on auklet reproduction may be less than demonstrated here, if females change sites and breed successfully in subsequent years. There is no information on competition for nesting burrows or success of birds after switching to different breeding areas. It is possible, however, that the overall negative effect of oil exposure is less than observed here in the years after dosing, as some of the birds which were never seen again may have switched sites and bred successfully with new mates.

D. Auklet Study III: Exposure to Oil During Incubation in 1984

1. 1984 Field Season

Dosing and disturbance of auklets at night was stopped March 31, 1984 in an attempt to encourage the birds to initiate breeding and increase the number of pairs occupying MMS boxes. A revised study plan was developed in late March and early April to work with all of the unclosed birds which might lay once disturbance was minimized. The revised plan called for dosing auklets externally with

TABLE VI-8. 1985 BREEDING SUCCESS OF 1984 PRE-LAY EXPERIMENTAL AUKLETS<

	CONTROL		ORAL		EXTERNAL	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
NO. RETURNING/TOTAL	3/7	1/7	5/8	0/8	7/17	5/17
%RETURNING	42.9	14.3	62.5	o	41.2	29.4
NO. BIRDS LAYING EGGS	3	1	5	0	6	5
% LAYING EGGS	100	100	100	0	85.7	100
NO. BIRDS HATCHING EGGS	3	1	3	0	3	3
HATCHING SUCCESS(%)	(loo)	(loo)	(60)		(50)	(60)
NO. BIRDS FLEDGING CHICKS	2	1	o	0	1	1
FLEDGING SUCCESS(%)	(67)	(loo)			(17)	(20)
NO. BIRDS RELAYING EGGS	o	o	0	0	1	1
NO. RELAYS HATCHED	0	0	0	0	0	0
RELAY HATCHING SUCCESS (%)						
NO. RELAYS FLEDGED	0	0	0	0	0	0
RELAY FLEDGING SUCCESS (%)						

weathered crude during incubation when they would be less likely to abandon because of their commitment to incubating the egg. Days 14 and 15 of incubation (one third of the total incubation time) were selected as a suitable time for dosing, because fecal samples had been obtained from birds on those days in previous years, and birds had not abandoned. Checking boxes and dosing birds on both Days 14 and 15 were necessary as only one bird remains in the box and incubates, but incubation is shared and auklets switch incubation duties nearly every night.

In order *to* increase the sample size to provide as many birds as possible for this study, the use of additional nest boxes was granted by PRBO. After consultation with PRBO and the U.S. Fish and Wildlife Service, San Francisco Bay National Wildlife Refuge, permission was granted to substitute 40 unused MMS boxes.

The additional boxes were designated "NOAA" boxes, because they had been installed in 1977 and 1978 for a National Oceanic and Atmospheric Administration (NOAA) Outer Continental Shelf, Environmental Program Study of Cassin's Auklets.

Many NOAA boxes had been used by the same birds over several years, and thus represented an established colony of breeding birds. The MMS boxes, on the other hand, had been used by fewer birds, and all of the experimental birds were banded and studied in the year they were first seen. Many of these birds may have been young or inconsistent breeders. Comparison of the two *sets* of breeding boxes, therefore, had the possibility of providing data on different responses of young birds, in contrast with established birds, to the disturbance of oil during the breeding season.

Birds in both MMS and NOAA boxes were banded during daylight on days 1 and 2 of incubation, and the boxes were not further disturbed until days 14 and 15 when incubating birds were measured, weighed, examined for molt, and dosed (or sham dosed and designated as controls). Birds were checked every 8 days after dosing (Days 22 and 23, 30 and 31, 38 and 39) during incubation for attendance, collection of a fecal sample, and examination of molt.

Pairs of birds were dosed in the 40 NOAA boxes which had birds on days 14 and 15 of incubation. The breeding records and growth rate data of 40 comparable NOAA nest boxes were provided as control data for the dosed birds. One or both birds in 7 of the 40 experimental NOAA boxes abandoned prior to day 14 and these boxes were excluded from dosing and excluded from all data analysis. Similarly, one or both of the birds in 12 of the 40 designated control boxes had abandoned prior to day 14 of incubation, and these boxes were also excluded from analysis. The two groups were treated similarly throughout the season so as to make direct comparisons, with the exception that the control birds in NOAA boxes were handled every 4 days to check for molt, while MMS controls and all dosed birds were handled every 8 days. The reason for this difference was that a PRBO biologist was studying molt patterns in birds in the NOAA boxes and permission was granted to use the data from those birds as NOAA controls. The results of the comparison of birds dosed on Day 14 or 15 of incubation in the MMS and NOAA boxes is given in Table VI-9.

Significant differences were obtained in abandonment and hatching success of dosed auklets as compared with controls. The high

Table VI-9. Fates of first and second nesting attempts by experimental auklets treated during incubation in 1984. Comparison of original "MMS" boxes and PRBO "NOAA" boxes *.

	"MMS" BOXES		"NOAA" BOXES	
	CONTROL	EXTERNAL DOSE DURING INCUBATION	CONTROL	EXTERNAL DOSE DURING INCUBATION
No. treated	32	34	56	66
No. birds abandoning first egg	8	31[a,c]	8	32[b]
No. eggs hatched	22	2	40	18
Hatching success (percent)	68. 8%	5.9%	71.4%	27.3%
No. fledge	16	2	28	16
Fledging success (percent)	72. 7%	100Z	70%	88. 9%
No. of second nesting attempts (relay eggs)	2	3	20	24
No. relays hatched	2	1	8	6
Hatching success (percent)	100%	33.3%	40%	25%
No. relays fledged	o	0	o	6
Net breeding success (No. chicks/bird)	0.500	0.059	0.500	0.333

*: Chi-square analysis using Yate's correction.
a: Significantly greater abandonment of MMS dosed compared to MMS Control birds ($p<0.01$).
b. Significantly greater abandonment of NOAA dosed compared to NOAA Control birds ($p<0.01$).
c. Significantly greater abandonment of MMS dosed compared to NOAA Dosed birds ($p<0.05$).

abandonment of dosed birds in MMS boxes (94.1%) is highly significant when compared to controls (25.0%), and the low hatching success and low net breeding success which followed is also significant. Significantly more of the dosed birds in NOAA boxes abandoned (48.5%), compared with NOAA controls (14.3%). Fewer of the established birds abandoned as a result of exposure to oil, and the NOAA controls abandoned less from disturbance than control birds in the MMS boxes, even though the NOAA controls were handled every 4 days and the MMS birds only every 8 days.

The number of re-laying attempts was very low for both dosed and control birds in MMS boxes. Birds in NOAA boxes, both dosed and controls, relaid with significantly higher frequency.

Comparing the MMS and NOAA boxes demonstrates differences in response to oil when based upon prior breeding experience. All birds dosed in MMS boxes in 1984 were pairs identified for the first time in 1984, while most of the birds nesting in NOAA boxes had occupied those boxes in previous seasons, some for as long as six years. Oil exposure caused breeding failure in both groups, but the experienced birds re-laid when their first egg failed. The frequency of re-laying was not different between NOAA controls and NOAA oil-dosed birds, indicating that both groups remained committed to the breeding season. Oil exposure, therefore, appears to cause greater disruption to inexperienced or marginally breeding birds than to established breeding birds.

Application of oil to adults during incubation made it inevitable that small amounts of weathered crude oil would be transferred to most eggs during incubation after dosing. Auklets were dosed on the central breast plumage, and the brood patches were deliberately avoided, but most of the incubating birds got smudges of oil on the surface of their eggs. Auklet brood patches are located on the lateral aspect of the chest and are termed "axillary" brood patches, but eggs were nevertheless oiled during incubation. We checked for and recorded the amounts of oil on incubating birds and on eggs each time the boxes were opened throughout incubation.

Twenty-two of 31 eggs, which were abandoned immediately after dosing in MMS boxes, had smudges of oil (Table VI-10). One egg which was incubated full-term, but did not hatch, was contaminated with oil, and the only two eggs that did hatch were smudged with oil. Trace amounts of oil were transferred to eggs in the majority of cases. The amounts of oil were subjectively assessed, recorded each time observed, and scored: 0 = no sign of oil; 1 = light smudge of color or a thin oily film with a faint smell of petroleum; or 2 = heavy smearing with black oil and a strong odor of petroleum. Only two eggs were scored as 2, both from MMS boxes, and were abandoned shortly after dosing. We attempted to determine the quantities of oil contaminating the eggs by applying measured quantities of oil to collected egg shells and spreading the oil into thin films *to* match the smudges on incubated eggs. These approximate determinations indicated that only two eggs received as much as 3 ul of oil from the incubating adults, and the quantities on most eggs were 1 ul or less.

Oil contamination of eggs causes embryo mortality and reduced matchability if eggs are contaminated early during incubation (Hoffman 1978, 1979; Szaro and Albers 1978; Albers 1978, Lewis and Malecki

TABLE VI-10. TRANSFER OF OIL FROM PLUMAGE TO EGGS DURING INCUBATION AND FATES OF EGGS*.

	MMS Boxes		NOAA Boxes	
	Controls	Dosed	Controls	Dosed
No. birds treated	32	34	56	66
No. oiled eggs		25		58
No. abandoned		22		29
No. failed to hatch		1		13
No. hatched		2		8[a]
Hatching success (%)		8%		14%
No. unoiled eggs	32	9	56	8
No. abandoned	8	9	8	3
No. failed to hatch	2	0	8	3
No. hatched	22	0	40	2
Hatching success (%)	69%		71%	25%

*: Chi-squared analysis using Yate's correction.

a Significantly fewer "NOAA" oiled eggs hatched than "NOAA" control eggs ($p<0.05$).

1983). Field data of abandonment and matchability were separated to differentiate between these two possibilities as causes of reduced hatching success. Most of the auklets abandoned shortly after being dosed, especially in MMS boxes. The hatching success of those birds which did not abandon was compared between treatment groups. The hatching success of eggs of oiled birds in NOAA boxes was lower (52.9%) than the success of full-term incubated eggs of unoiled controls (83.3%) but there were too few eggs to make the differences significant. The number of MMS birds attempting to incubate to full term was too low for statistical analysis. The high rate of abandonment combined with reduced matchability of eggs of oiled birds combined to result in very high embryo mortality, regardless of the possible direct embryo toxicity of oil. Most abandoned eggs were broken in the nest. All exposures of 1-5 ml produced lowered hatching success, but the quantities of oil transferred to eggs and embryo mortality were not directly determined.

2. 1985 Field Season

Approximately half of all oil-exposed auklets as well as controls returned to breed in 1985. NOAA birds returned with equal frequency whether dosed or control, but a lower percentage of the MMS dosed birds returned, although the difference compared to MMS controls is not significant. The data for returning birds are presented in Table VI-11 organized by treatment group and in Table VI-12 separated by sex. The trend of lower returns by MMS dosed birds is the same as in 1983 and 1984 after dosing in 1982, and is a result of a larger proportion of females not returning to MMS boxes, a trend repeated for the third time in three studies. The return to NOAA boxes was independent of sex in both control and dosed groups. The laying frequency, hatching success, fledging success, and net breeding success of returning birds in NOAA boxes was similar for all four groups.

Table VI-13 separates the data further into birds which changed mates in 1985 compared to those which remained with their mates of 1984. Dosed birds in both MMS and NOAA boxes changed mates' more frequently than did control birds (85% vs. 53% inMMS [$p < 0.05$]; 76% vs. 61% in NOAA [n.5]), and the hatching and fledging success of birds which switched mates was lower than for pairs of birds which remained together ($p < 0.05$). In every treatment group, however, when returning pairs are compared to those which changed mates, the group of returning pairs had greater net breeding success than the new pairs. The re-laying frequency of birds which remained with the same mate is also significantly higher, reflecting the results of NOAA and MMS re-lays in 1984, where established birds relaid with significantly higher frequency than birds in MMS boxes.

E. Summary and Discussion of Auklet Field Studies

Three separate field experiments were conducted with Cassin's Auklets from 1982 - 1985. In each study, auklets were dosed with 1 ml weathered Santa Barbara crude oil applied externally on the breast plumage or orally by gelatin capsule, and each group was compared to a group of control auklets.

A high proportion of auklets dosed externally with oil prior to egg laying responded by abandoning the breeding season. Those birds continuing to breed were delayed in egg laying by more than 20 days in both the 1982 and 1984 studies. The delay appeared to be a consequence of disruption of egg formation and probably delay in the initiation of growth of new ovarian

Table VI-11. 1985 Breeding Success of 1984 Post-Lay Experimental Auklets.

	"MMS" Boxes		"NOAA" Boxes	
1985 Fate	Control	Post-Lay External Dose	Control	Post-Lay External Dose
No. Returning/Total	18/32	14/34	29/56	34/66
% Returning in 1985	53%	41%	52%	50%
# birds laying eggs	17	14	29	33
% birds laying eggs	94%	100%	100%	97%
# birds hatching eggs	12	11	24	30
Hatching success (%)	71%	79%	83%	91%
# birds fledging chicks	11	9	19	29
Fledging success (%)	92%	82%	79%	97%
# birds relaying eggs	4	3	5	2
# birds hatching 2nd egg	1	2	1	0
Relay hatching success (%)	25%	67%	20%	0
# birds fledging 2nd egg	1	1	1	
Relay fledging success (%)	100%	50%	100%	
Net breeding success (No. chicks/adult laying)	.71	.71	.69	.88

TABLE VI-12: 1985 BREEDING SUCCESS BY SEX OF 1984 POST-LAY EXPERIMENTAL AUKLETS

	MMS BOXES				NOAA BOXES			
	CONTROL		EXTERNALLY DOSED IN 1984		CONTROL		EXTERNALLY DOSED IN 1984	
	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
NO. RETURNING/TOTAL	10/16	8/16	10/17	4/17	15/28	14/28	15/33	19/33
% RETURNING	63%	50%	59%	24%	54%	50%	45%	58%
NO. BIRDS LAYING EGGS	9	8	10	4	15	14	15	18
% LAYING EGGS	90%	100%	100%	100%	100%	100%	100%	95%
NO. BIRDS HATCHING EGGS	8	4	8	3	13	11	13	17
HATCHING SUCCESS (%)	89%	50%	80%	75%	87%	79%	87%	94%
NO. BIRDS FLEDGING CHICKS	7	4	6	3	11	8	12	17
FLEDGING SUCCESS (%)	88%	100%	75%	100Z	85%	73%	92%	1 00%
NO. BIRDS RELAYING EGGS	1	3	2	1	2	3	2	0
NO. RELAYS HATCHED	0	1	1	1	1	0	0	0
RELAY HATCHING SUCCESS (%)		33%	50%	100%	50%			
NO. RELAYS FLEDGED	0	1	1	1	1	0	0	0
RELAY FLEDGING SUCCESS (%)		100%	1 00%	100%	100%		0	0

TABLE VI-13. 1985 MATE SWITCHING OF 1984 POST-LAY EXPERIMENTAL AUKLETS.

	MMS BOXES				NOAA BOXES			
	CONTROL		POST-LAY EXTERNAL DOSE		CONTROL		POST-LAY EXTERNAL DOSE	
	SAME MATE	NEW MATE	SAME MATE	NEW MATE	SAME MATE	NEW MATE	SAME MATE	NEW MATE
No. returning and laying eggs	8	9	2	11	11	17	8	25
% Birds Switching Mates		53%		85%		61%		76%
No. birds Hatching chicks	7	5	2	9	10	14	8	22
Hatching Success (%)	88%[a]	56%	1 00%	82%	91%	82%	100%	88%
No. birds fledging chicks	6	5	2	7	10	9	8	21
Fledging Success (%)	86%	100%	100%	78%	100%	64%	100%	95%
No. birds Relaying eggs	4	0	1	2	5	0	2	0
No. birds Hatching 2nd egg	1	0	0	2	1	0	0	0
Relay Hatching Success (%)	25%	-		100%	20%	–	-	
No. birds fledging 2nd chick	1	0	0	2	1	0	0	0
Relay Fledging Success (%)	100%	-		100Z	100%	-	-	

a. Overall hatching and fledging success of same mate pairs was significantly greater than new mate pairs for both MMS and NOAA birds ($p<0.0195$; Sign test).

follicles. In 1984, an experimental group was dosed orally with 1 ml of oil in gelatin capsules. The results were less severe than with external exposure, with only a short delay in egg laying, and no effect on laying frequency, hatching success, or fledging success.

The results observed here are consistent with reports in the literature of interference with reproduction, suggesting breeding depression as a result of stress and direct impairment of egg formation in females. Chronic feeding of South Louisiana, Kuwait crude, or component fractions to ducks has resulted in delay of ovarian maturation, altered levels of ovarian hormones, decreased intensity of lay, decreased egg shell thickness (Holmes et al. 1978; Vangilder and Peterle, 1980; Coon and Dieter, 1981; Harvey et al. 1981; Cavanaugh and Holmes, 1982; Cavanaugh et al. 1983), and follicular atresia (Holmes et al. 1978). Single oral doses of Bunker C or Prudhoe Bay crude caused short-term disruption of follicular development and abnormal yolk formation, and caused reduced matchability in quail (Grau, et al. 1977; Wootton et al. 1979). Cassin's Auklets were inhibited in initiation of rapid yolk formation by single oral doses of Bunker C, and birds which laid eggs had a reduced commitment to incubate (Ainley et al. 1979, 1981). A finding expanded upon in recent studies by Cavanaugh et al (1983) and Harvey et al (1982) has demonstrated decreases in circulating prolactin were correlated with altered incubation behavior. Ingestion of Prudhoe Bay crude depresses food intake or foraging which secondarily results in lowered egg production (Engel et al. 1978) or reduced growth and survival of chicks if adult birds are contaminated during the hatchling period (Travelpiece et al. 1984). The difficulties of interpreting primary and secondary effects on reproduction are discussed by Harvey et al. (1982) and results are complicated by depressed growth, altered hormone metabolism, stress, and probable central nervous system effects. The short review by Albers (1983) contains a useful summary table of all studies through 1982, which investigated effects on reproduction.

Returning Cassin's Auklets were observed for 1-3 years after dosing. Fewer dosed females returned to breed in subsequent years, but the return of males was not influenced by oil exposure. Females may have moved to other areas on SEFI, but none were ever observed in nest boxes or monitored burrows on other parts of the island.

Oil exposure caused greater abandonment of pairs breeding for the first time in nest boxes than for established pairs. Re-laying frequency of both dosed and control pairs was higher in the established boxes.

Oil exposure resulted in a lower proportion of females returning and a higher proportion of dosed birds changing mates in the year following exposure. Changing mates resulted in lowered success in hatching and fledging the first egg, reduced relaying attempts, and lowered net breeding success in the year after exposure.

In this study, auklets were exposed externally with 1 ml of oil on day 14 or 15 of incubation, resulting in a high frequency of abandonment, low hatching success, and low net breeding success of exposed birds. Eggs which became oiled during incubation, and were incubated to full term, had lowered hatching success, indicating the possibility of direct embryo toxicity of oil transferred to eggs by incubating adults.

A review and bibliography of all available studies of the effects of oil on eggs including short summaries of the original data has been prepared and included as Appendix B.

The review of the literature indicates that very small amounts of some crude oils are embryotoxic, with as little as 1-5 ul of South Louisiana, Prudhoe Bay or Kuwait crudes killing a majority of embryos in treated eggs. The age of embryos at dosing is a mitigating factor, with older embryos showing greater survival (Szaro, 1977; Albers, 1978). More than 90% of mallard embryos younger than 50% of term were killed by 5 ul of South Louisiana crude, while only 12% of the embryos in eggs dosed at 18 days of incubation (64% of term) failed to hatch. Weathering of crude oil also has a slight mitigating effect, with increased survival of mallard embryos exposed to small amounts of weathered Prudhoe Bay (Szaro et al. 1980) or Libyan crudes (Macko and King, 1980). Controlled field studies of oil exposed adults transferring oil to eggs have been made by King and Lefever (1979) with Laughing Gulls dosed with No. 2 Fuel Oil, Albers (1980) with Mallards exposed to Prudhoe Bay crude, and Lewis and Malecki (1983) who applied Kuwait crude or No. 2 Fuel Oil to Great Black Backed and Herring Gull brood patches. In this study, the amount of oil transferred to eggs during mid-incubation was 3 ul or less, quantities likely to produce threshold effects on embryo survival. The number of eggs exposed in this Study, however, was too small to provide a statistically significant effect.

VII . FIELD STUDIES TO DETERMINE THE EFFECT OF OIL ON WEDGE-TAILED SHEARWATERS

Wedge-tailed shearwaters were the second procellariid species selected for preliminary studies in 1982. These species are not common in California waters, but they are closely related to Sooty shearwaters and are the most accessible shearwater species breeding in US waters.

The present study investigated a free-living population of Wedge-tailed shearwaters (Puffinus pacificus) exposed to small, single, doses of weathered Santa Barbara crude oil early in the courtship phase of the 1983 and 1984 breeding seasons. The breeding success of exposed and control birds was evaluated throughout the breeding seasons of 1983, 1984 and 1985.

A. Field Site and Breeding Chronology on Manana Island

The study site was a Wedge-tailed shearwater breeding colony of more than 20,000 birds nesting in burrows dug into volcanic tuff soil on Manana Island, offshore from Oahu, Hawaii (Shallenberger 1973). Shearwaters begin burrow excavation and courtship in March and April. Successful breeding pairs regularly return to the same or an adjacent burrow each year. Shearwaters spend much time at sea during the day and return to the colony at nightfall throughout April and May. Pairs leave the colony in late May and June for a pre-laying exodus of 10-30 days, and soon after returning, the female lays a single egg. During the exodus period the burrows of many pairs in the study plot became occupied by other shearwaters, and returning pairs often discovered another pair incubating an egg. The returning pair either displaced the incubating bird, laid an egg and pushed the other egg from the burrow, or they were prevented from reoccupying the burrow and moved to a burrow nearby to lay and incubate their egg. The incubation period is 48-52 days with males and females alternating incubation intervals of 4-7 days. However, eggs may be left unattended for several days resulting in arrested development when eggs cool (Whittow 1980; Ackerman et al 1980). The first eggs are laid in early June. Chicks hatch during August at a weight of about 40 g and grow with a relatively constant increase in weight each day for the first 7-10 days. Feeding intervals become variable after the first week, and the increases in weight become erratic. Chicks reach adult weights of about 400 g at 60 days and fledge in November at 120-150% of adult weight.

B. Methods

Manana Island was visited 5 times during the period May 27 to June 12, 1982, in order to band shearwaters, mark nesting burrows and collect blood samples and eggs.

Burrows were marked with numbered redwood lath stakes driven into the ground adjacent to the burrow entrance. Lath stakes worked well and most stakes lasted for all 4 seasons.

1. Determination of Egg Formation Time

The timing of egg formation and correlation with the pre-laying exodus was determined in order to evaluate possible effects of oil dosing. In late May 1982, the year before the oil study, twenty pairs of shearwaters were banded and fed capsules containing 100 mg Sudan Black B to identify yolk deposition during egg formation (Grau 1976). Six eggs of dye-fed birds were collected June 25, and the timing and duration of yolk formation were studied by freezing the eggs, fixing yolks in formalin, and staining with bichromate.

2. Field Protocol

In early May 1983, a study plot of 60 m X 80 m was selected on the southwest side of Manana Island. A grid with a spacing of 2 m X 3 m was marked with stakes to identify coordinantes of burrows within the plot. Occupied burrows were marked with numbered stakes. Burrows were checked every two or three days during the period May 25-June 11, 1983 and were marked if found to be occupied. Most shearwater burrows in the plot were 0.5-1.0 m long, although some were too deep for this Study. During the incubation period (June 12-August 15, 1983) burrows in the study area were checked weekly to locate birds which had returned from their exoduses.

Mated pairs were selected on the basis of their courtship behavior (Shallenberger 1973 and pers comm). Mated pairs spend much time together deep in the burrow during the night and early morning hours; while unmated, courting birds rest facing each other at the burrow entrance. Only mated pairs deep in burrows were selected. Pairs were banded with stainless-steel bands on both legs to minimize identity loss. All birds were weighed to the nearest gram with a spring balance and 168 were measured (tarsus length, culmen length, and nares to bill tip length) to determine body size variability and evidence of sexual dimorphism. Six pairs of non-experimental birds were examined by laparoscopy to determine sex; body-size dimorphism was not found to be a dependable indicator. A female could be identified with certainty only during the week immediately after laying by her stretched and partially everted cloaca (Seventy 1956; Shallenberger 1973).

3. 1983 Oil Treatment

In 1983, 236 mated pairs of birds were assigned alternately during banding as controls, pairs to be dosed orally, or pairs to be treated externally with 2 ml of weathered oil. Pairs of birds were treated identically when banded and dosed. Sixty one pairs were examined, banded, measured, and weighed, then dosed orally with 2 ml weathered oil in two No. 0 gelatin capsules. Sixty pairs were oiled externally by spreading 2 ml weathered oil evenly over the central breast plumage. One hundred and fifteen pairs-of controls were examined in the same way, but were not further sham-dosed. A few days after dosing, some treated birds were discovered in burrows, each with an unhanded bird. In such cases, the unhanded bird was banded and treated exactly as the banded bird, either dosed or assigned as a control. An additional 92 pairs of unhanded birds laid eggs in marked burrows during the 1983 study and were used as a secondary control group. These birds were banded when discovered incubating an egg, but were not further disturbed during incubation except to band the mate after an incubation relief.

Oil dosing was conducted June 4-11, 1983, timed to precede the birds' departure on the pre-laying exodus. The time of dosing allowed us to observe: (a) the effects of oil on delay or interruption of egg formation; (b) the effect of stress on strength of pair bonds; and, (c) the nest site tenacity of returning birds. Marked burrows were checked at 2-3 day intervals during June and July for the return of banded birds and to determine date of laying. All eggs were marked when first located with burrow number and date. Marker stakes of burrows with incubating birds were painted to identify burrows that would not be disturbed during subsequent burrow checks. Our intent

was to obtain the most reliable data on lay and incubation with the minimum disturbance to incubating birds.

Hatching success was determined by checking burrows 56 days after the discovery of the egg, thus avoiding disturbance of late hatching chicks. Eggs not hatched at 56 days were candled with an otoscope light and if the embryo was dead, the egg was collected. Empty burrows were searched for egg shell fragments to discover whether the egg was lost during incubation or the chick lost after hatching.

4. 1984 Oil Treatment

A second experiment was conducted in 1984 following the procedures of 1983, but using 1.0, 0.5 or 0.1 ml of weathered crude oil applied externally to the breast plumage of both members of a pair. Thirty pairs of previously unclosed shearwaters were dosed at each level of oil during the period May 20 to June 6, 1984. In 1984, 51 returning control shearwaters banded in 1983 were located before the pre-laying exodus and these birds were followed as controls for the 1984 oiled birds and throughout the 1984 and 1985 breeding seasons. Thirty-six additional 1983 controls were located during incubation in 1984.

5. Growth Rates of Chicks

Growth rates of chicks were measured from hatching through October 1, 1983 (chick ages of 35 to 70 days) by weighing chicks with a calibrated spring balance every 4-7 days. Because of variations in incubation periods and uncertainty of date of lay, hatching dates were only known within $\pm$ 2 days. Young chicks were brooded by adults only during the first week after hatching. Handling chicks was avoided when adults were present, because adults with chicks regurgitated food when disturbed. Hatching weights and daily growth rates of 12 chicks from unmarked burrows were determined during the first 15 days. Chick weights for the first week were found to be predictive of age. Fledging success was not determined. Chicks older than 40 days occasionally moved from burrow to burrow and could not be identified with certainty, because in 1983 chicks were not banded with temporary bands.

Chick rearing was termed successful if chicks reached 250 g in weight or were alive on October 1.

6. Analysis of Breeding Data

Breeding activity and success were scored on an individual bird basis. Mates of some birds were not identified when incubating, because burrows were checked only 3 or 4 times during incubation to prevent abandonment. Birds which switched mates in the second year were tabulated in their original treatment group.

Original data were transferred to a database management program (dBASE II) for tabulation of results with a microcomputer. The breeding success of each bird during 1983 was compared with its breeding success in 1984 and 1985 and compiled by treatment group. Treatment groups were compared and differences assessed using one-way analysis of variance for treatment groups of unequal sizes. Burrow switching data and distances to new burrows were compared between groups using the t distribution. Chi square and Fishers Exact tests were used *to* assess the significance of differences between compared groups (Zar, 1984) using a statistical calculation program (Statpak, Northwest Analytical Inc., Portland, OR) on a microcomputer.

c. Results

1. Egg Formation Period

Yolks (N=6) contained 17-19 yolk rings plus an unstained central core of 5-7 mm diameter, signifying a yolk formation time of 21-23 days (Figure VII-1) (see Grau 1984 for an explanation of yolk ring staining and correlation of daily yolk deposition). Only one egg contained Sudan black dye. This was confined to the central core of the yolk, indicating that in late May birds had not yet initiated rapid yolk deposition, and that the egg was formed during the pre-laying exodus. This is consistent with laparoscopy findings, which indicated that none of the five females examined on June 1, 1983 had follicles larger than 5 mm. There may be variation in the length of pre-laying exodus and in the time required for egg formation in different Wedge-tailed shearwater colonies. Roberts et al. (1974) reported that some Australian shearwaters had follicles up to 15 mm diameter just before the pre-laying exodus, representing ova undergoing rapid yolk deposition.

2. Effect of Oil on Pre-laying Exodus and Return to Burrow, 1983

Treating shearwaters with 2 ml of weathered crude oil had no effect on the length of the pre-laying exodus and did not cause birds to move to new burrows. All birds left the colony for a pre-laying exodus of about 28 days soon after being banded and treated with oil in 1983. The average duration of the exodus was not statistically different between control and treated groups (Table VII-l). A record was made of all burrow switching and distances from old to new burrows, because treated birds might have returned to a different part of the colony as an avoidance reaction after being treated (Table VII-2). Eighty nine percent of birds from both control and oil treated groups returned to their original or an adjacent burrow. Externally oiled birds returned with a much lower frequency (21%) than orally dosed or control birds but they all returned to burrows within 2-3 m of the burrow in which they were exposed to oil. Four externally oiled shearwaters located in the colony 2, 7, or 9 days post-oiling were partially or completely cleaned and their plumage was undamaged. All externally dosed birds which returned to the colony to lay after the pre-laying exodus had clean plumage.

3. Egg Laying and Incubation, 1983

Fifty-four percent of the control birds were observed incubating eggs (Table VII-3). Fifty percent of their eggs hatched and 65% of the chicks survived. Thirty-two percent of the orally dosed birds incubated eggs; thirty-eight percent of these eggs hatched and 53% of the chicks survived. The laying and incubation frequency of the orally dosed birds was significantly lower than controls or pairs of non-experimental birds which were banded during incubation. Twelve percent of the externally oiled birds were observed incubating eggs. This was significantly lower than incubation frequency of either control or orally dosed birds. No eggs of externally oiled birds hatched. Five of the nine eggs laid by externally oiled birds were incubated by only one bird of the pair. All of these eggs were left unattended for 3-4 days at a time while the bird went to sea to feed, and all were broken early in incubation.

Unhanded birds laid eggs in marked burrows throughout June and July. The hatching success did not significantly differ for eggs laid early or late in the season. Banded birds began to lay later than unhanded

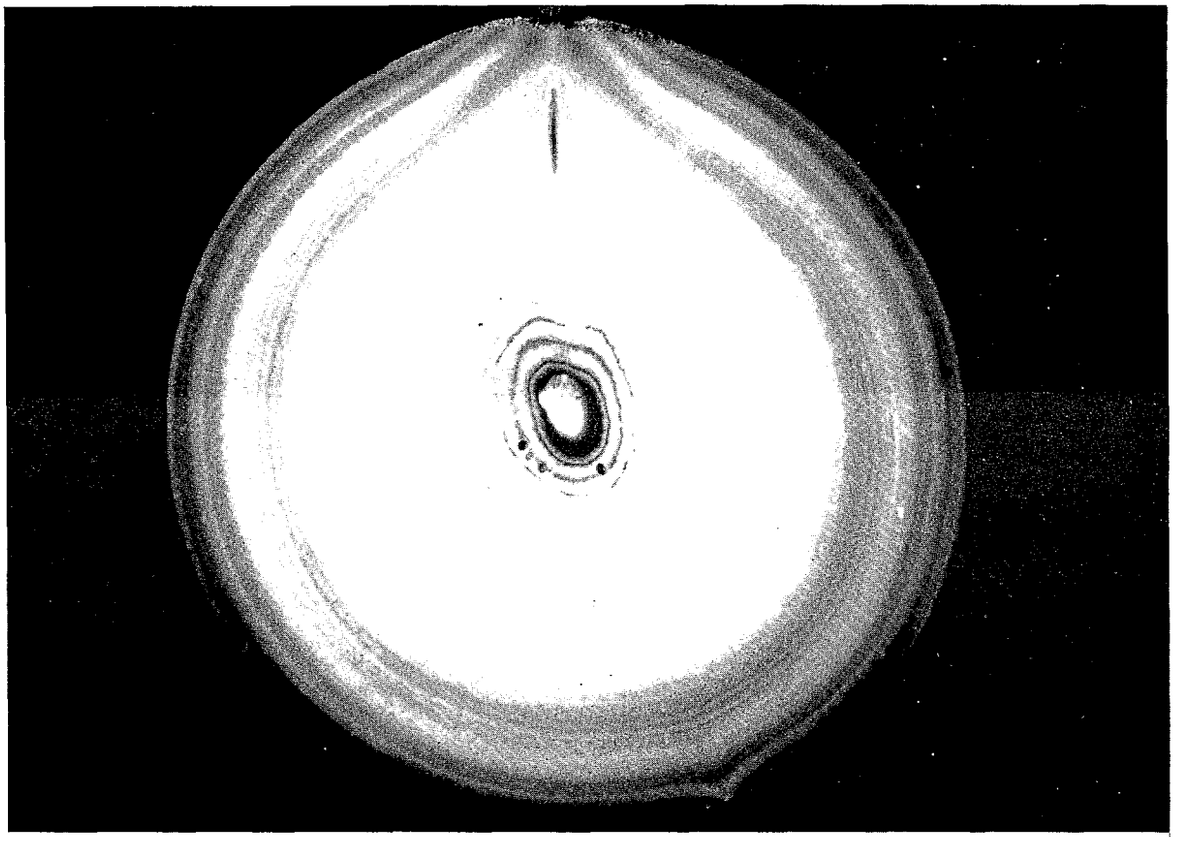


Figure VII-1. Wedge-tailed Shearwater egg yolk stained with potassium bichromate to demonstrate daily yolk depostion as evidenced by 19 concentric rings plus an unstained central core. This corresponds to a 21-23 day yolk formation time.

Table VII-1. Effect of weathered Santa Barbara crude oil on the length of Pre-laying exodus in Wedge-tailed Shearwaters.

Treatment Group*	Pre-laying exodus length (days + sem)	
	1983	1984
Control	29.5 ± 0.68[a] n=122	24.0 ± 2.37[a] n=20
Oral (2 ml)	27.6 ± 0.80 n=36	24.6 ± 2.25 n=11
External (2 ml)	27.8 ± 2.47 n=14	24.7 ± 1.47 n=11
External (1 ml)		25.1 ± 1.85 n=18
External (0.5 ml)		29.7 ± 2.29 n=21
External (0.1 ml)		28.2 ± 2.39 n=24

* One-way analysis of variance

[a] Values for length of exodus of controls are significantly different for 1583 and 1984 ($P<0.05$). No other pairs of values are different from each other.

Table VII-2. Burrow fidelity of Wedge-tailed Shearwaters exposed to weathered crude oil: Return of shearwaters in the year of exposure and the subsequent year.

	Number Returning	Return to Same Coordinate*		Return to Different Coordinate*		Average Distance to New Burrow**
Returns from 1983 Dose Burrow to 1983 Breeding Burrow:		#	%	#	%	
Control	150/230	133	89	17	11	5.6 m
Oral 2 ml	57/126	51	89	6	11	6.7 m
Ext 2 ml	21/121	21	100	0	0	---
Returns from 1984 Dose Burrow to 1984 Breeding Burrow:		#	%	#	%	
Ext 1.0 ml	30/60	26	87	4	13	6.4 m
Ext 0.5 ml	30/64	27	90	3	10	3.8 m
Ext 0.1 ml	32/60	30	94	2	6	5.4 m
Returns from 1983 Breeding Burrow to 1984 Breeding Burrow:		#	%	#	%	
Control	87/230	26	30[a]	61	70[a]	8.2 m[d]
Oral 2 ml	46/126	8	17[b]	38	83[b]	7.7 m
Ext 2 ml	30/121	6	20[c]	24	80[c]	9.6 m

* Chi-squared analysis

** Student's t test

a. Significantly different from 1983 Controls ($p<.001$).

b. Significantly different from 1983 Orals ($p<.001$).

c. Significantly different from 1983 Externals ($p<.001$).

d: Significantly different from 1983 Controls ($p<.05$).

Table VII-3. Wedge-tailed Shearwaters exposure to weathered crude oil: 1983 effect of oil exposure on breeding success.

Dose *	# Birds	Birds Returning		Birds Incubating		Hatching Success		Chicks Raised		Net Breeding Success	
			%[a]		%		%[b]		%		%[c]
0 (Control)	230	150	65	125	54	62	50	40	65	17	
2 ml Oral dose	126	57	45[e]	40	32 [e]	15	38	8	53	6d	
2 ml External dose	121	22	18[e,g]	14	12[e,g]	0	0[e,f]	0	0[e,g]	0[e,f]	

* Chi-squared analysis

[a] % Birds Returning is the number returning after exodus in 1983 divided by the total number in the group in 1983.

[b] % Hatching Success is the number of incubating birds which hatched chicks divided by the total number of birds in the group incubating eggs in 1983.

[c] Net Breeding Success is the number of chicks raised in 1983 divided by the total 1983 group size.

': Significantly different from controls (P<.01).

': Significantly different from controls (P<. 001).

': Significantly different from oral dose group (P<.01).

g. Significantly different from oral dose group (P<.001).

birds as a consequence of the timing of banding control and dosed pairs. We began banding on May 25 after some early laying pairs had evidently already left the colony on their pre-laying exodus. The laying dates of each of the experimental groups is presented in Figure VII-2. Figures VII-3, 4, 5, and 6 present the laying dates and hatching success of all groups and show that there were no differences in hatching success between early and late laying pairs.

4. Chick Hatching and Growth. 1983

The weights of all newly hatched chicks were similar (39g to 43g), regardless of treatment group. The weights of chicks older than 10 days were highly variable (Figures VII-7, 8, 9), as older chicks were apparently fed large meals only every few days when adults returned to the island (Pettit et al. 1984). The variations in weights of chicks of both dosed and control birds were similar, and the overall growth rates of chicks in each treatment group were not different. The survival of chicks of orally dosed birds was, however, significantly lower than that of controls.

5. Effect of Oil on Pre-laying Exodus and Return to Burrow, 1984

Birds, newly banded and externally oiled in 1984, left the colony for a pre-laying exodus duration of 25-3'0 days, with no differences between the treatment groups (Table VII-l). Birds did not switch burrows when returning from exodus after being dosed in 1984. Approximately 90% of all returning oiled birds laid their egg in the burrow in which they were treated or in an adjacent burrow within the same grid coordinate (Table VII-2). All 1983 birds returning in 1984 took slightly shorter exoduses in 1984 than in 1983.

6. Egg Laying and Incubation. 1984

The application to shearwaters of as little as 0.1 ml weathered crude oil 30 days before laying resulted in fewer eggs being laid and in lowered breeding success (Table VII-4). The laying frequency, hatching success and overall breeding success progressively declined as the oil dose was increased. The control birds observed in 1984 had almost identical breeding success to the controls of 1983, with 53% returning after exodus to incubate eggs. Fifty-nine percent of control eggs hatched and 75% of the chicks survived. Forty percent of the 0.1 ml externally oiled group incubated eggs, while 33% of the 0.5 ml treatment group incubated eggs, and only 27% of the 1.0 ml treatment group incubated eggs. The composite dose-response data from 1983 and 1984 revealed a nearly linear correlation between the dose and the percent of birds hatching eggs (correlation coefficient=0.94) (Figure VII-10).

7. Multi-year Effects of Oil Exposure on Breeding Success

Long term effects of oil exposure were assessed by following the breeding success of dosed and control shearwaters returning to the breeding colony one or two years after dosing. Many birds changed burrows between 1983 and 1984. Only 20-30% of birds banded in 1983 returned to a burrow within the same grid coordinate in 1984 (Table VII-2) . Most birds still returned to the same area of the colony, however, as the average distance to a new burrow was less than 10 m for all treatment groups, and there was no statistical difference between treatment groups in the distance moved to new burrows. Since the study area was a 60 X 80 m plot, it was possible to search eve ry burrow within the plot and locate most of the birds which laid and

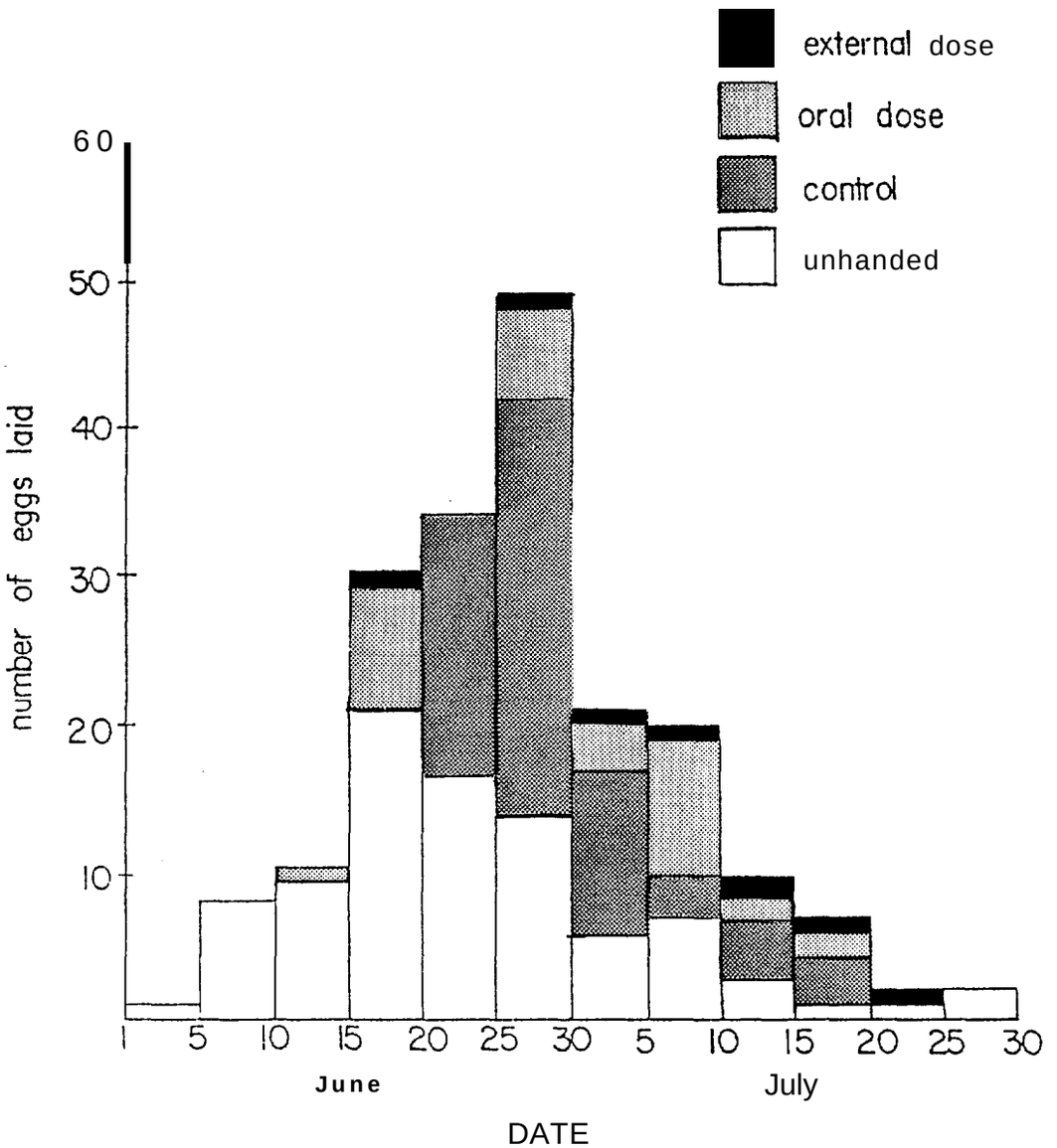


Figure VII-2. Laying dates and frequencies of control and experimental Wedge-tailed Shearwaters, 1983.

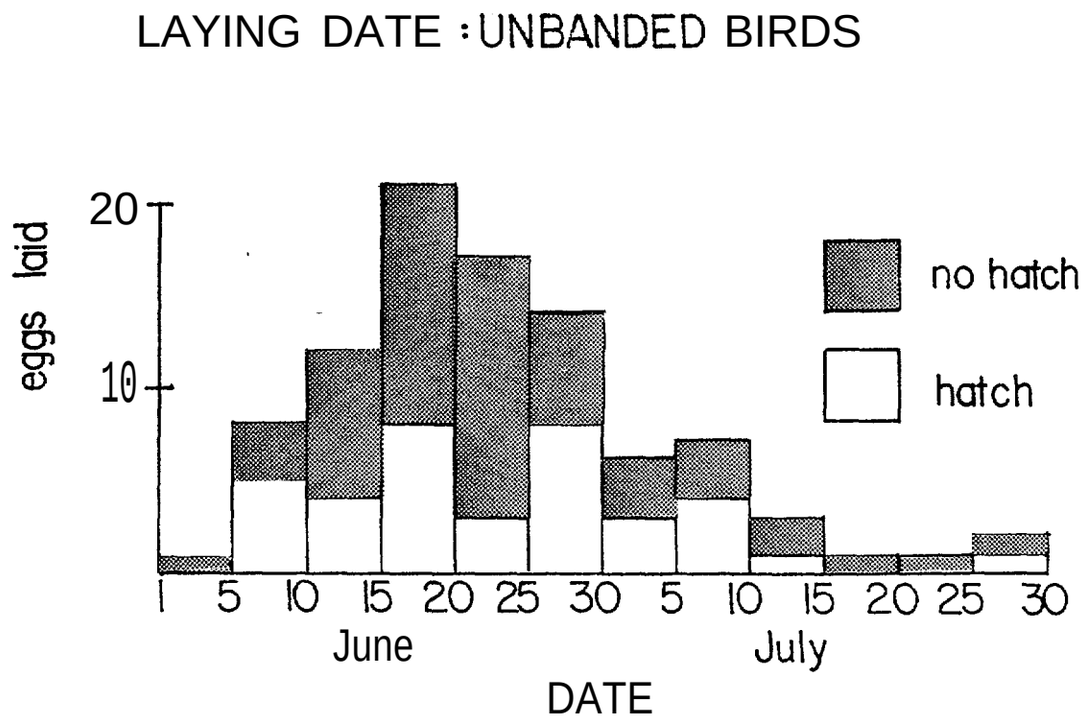


Figure VII-3. Laying dates and hatching frequencies of non-experimental Wedge-tailed Shearwaters, 1983.

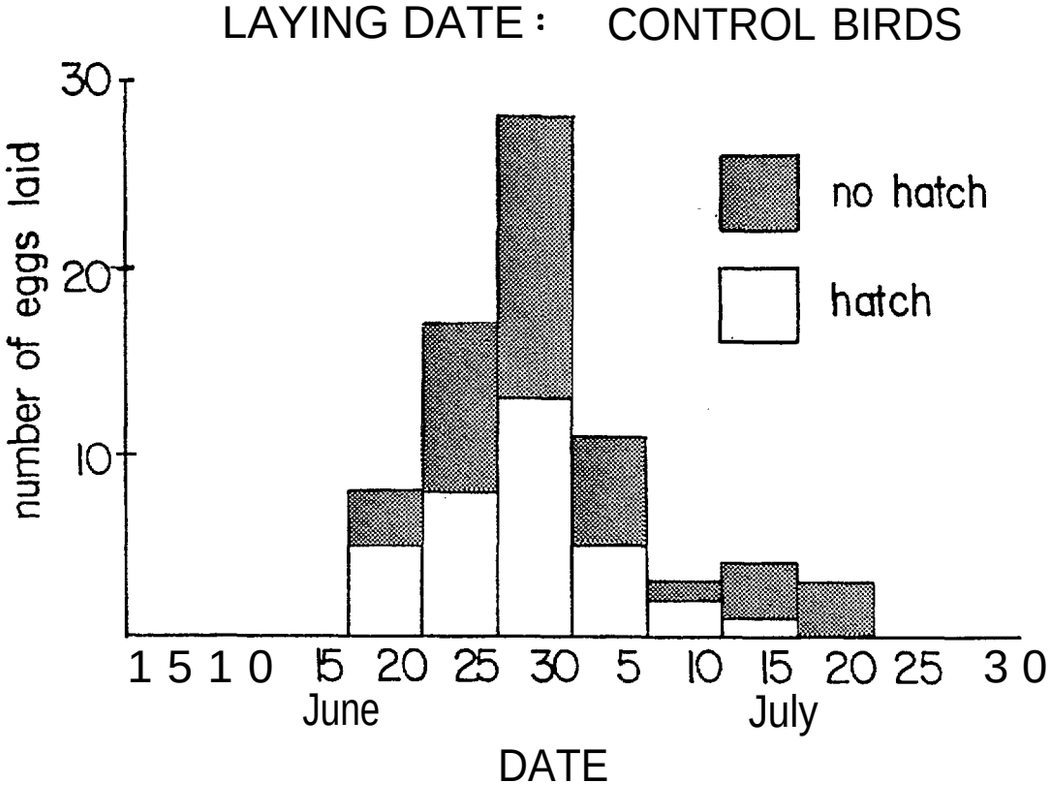


Figure VII-4. Laying dates and hatching frequencies of control Wedge-tailed Shearwaters, 1983.

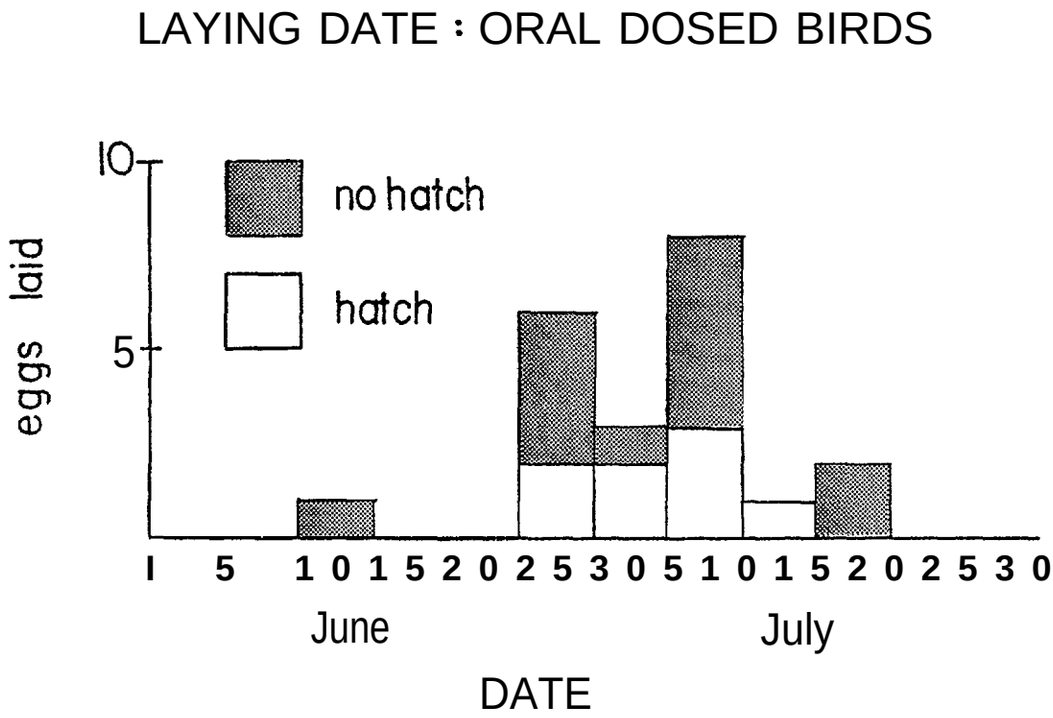


Figure VII-5. Laying dates and hatching frequencies of oral dosed Wedge-tailed Shearwaters, 1983.

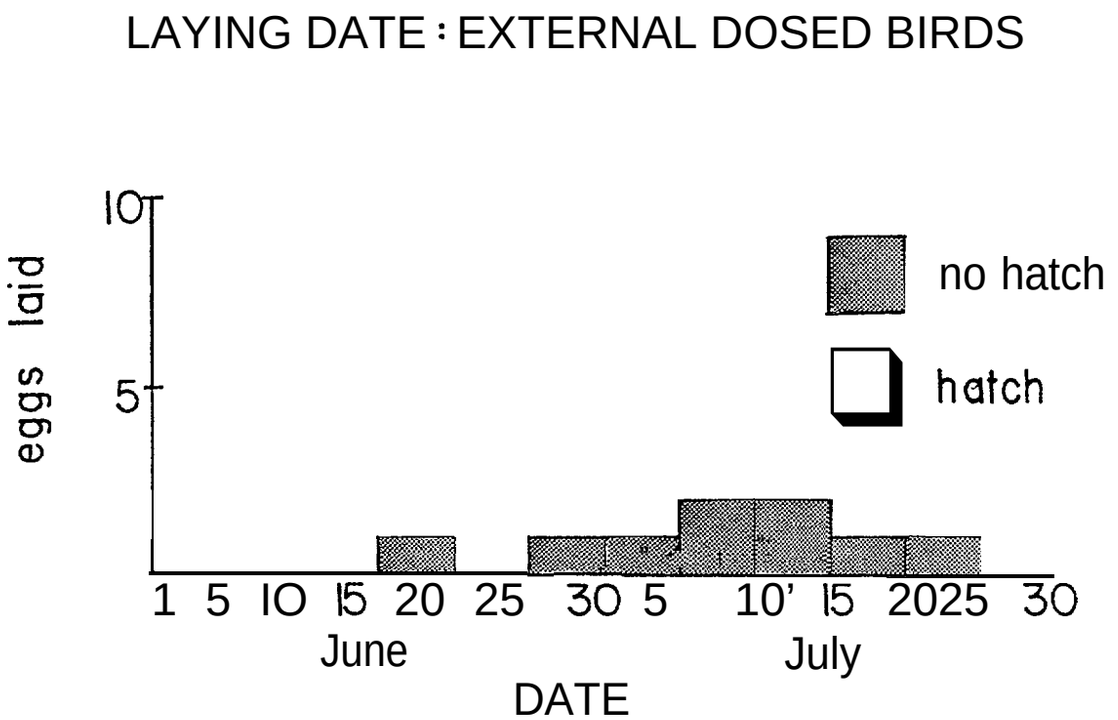


Figure VII-6. Laying dates and hatching frequencies of external dosed Wedge-tailed Shearwaters, 1983.

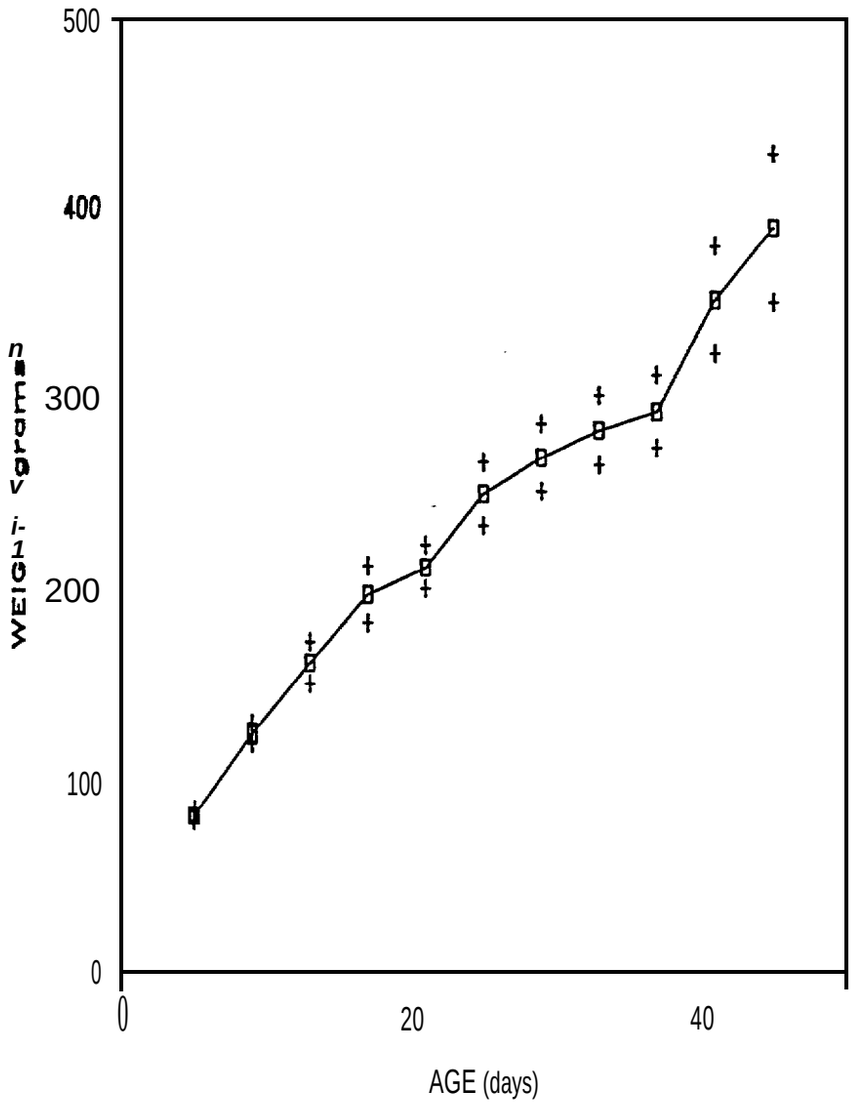


Figure VII-7. Growth rates of Wedge-tailed Shear water chicks from 1983 Control birds (n=12)(mean ± SE M).

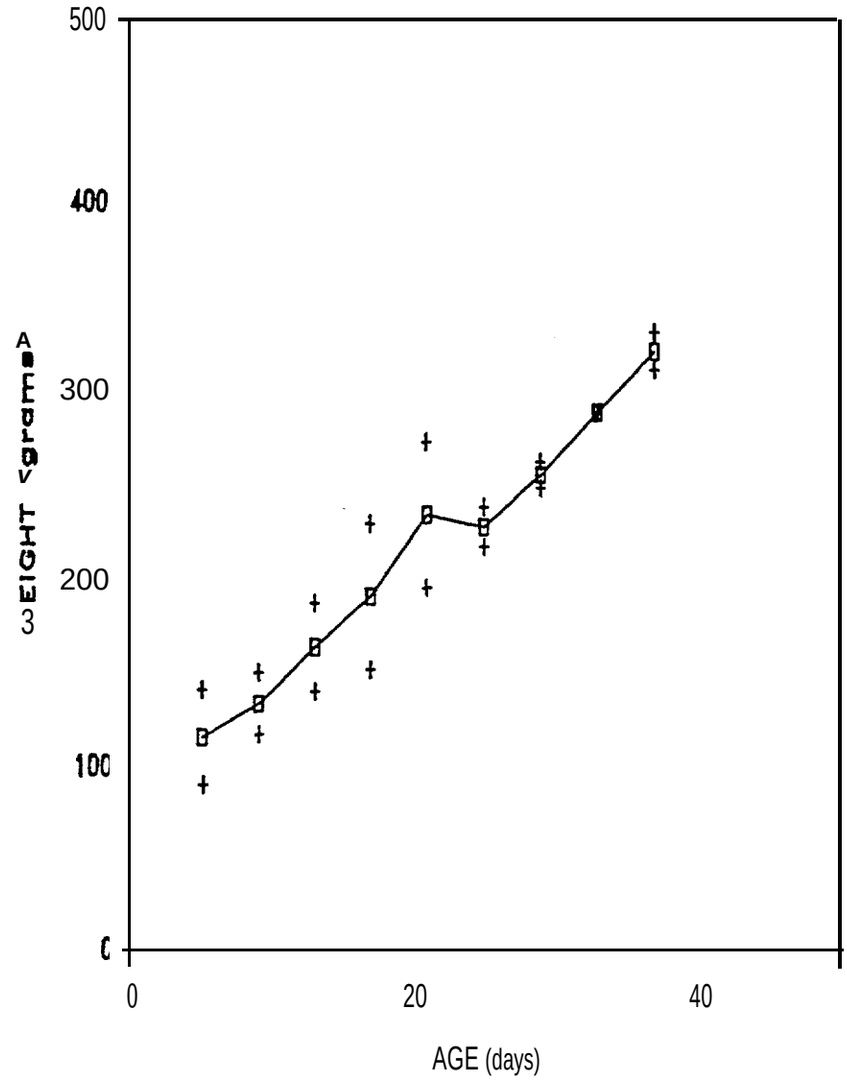


Figure VII-8. Growth rates of Wedge-tailed S hear water chicks from 1983 Oral dosed birds (n=4) (mean ± SE M).

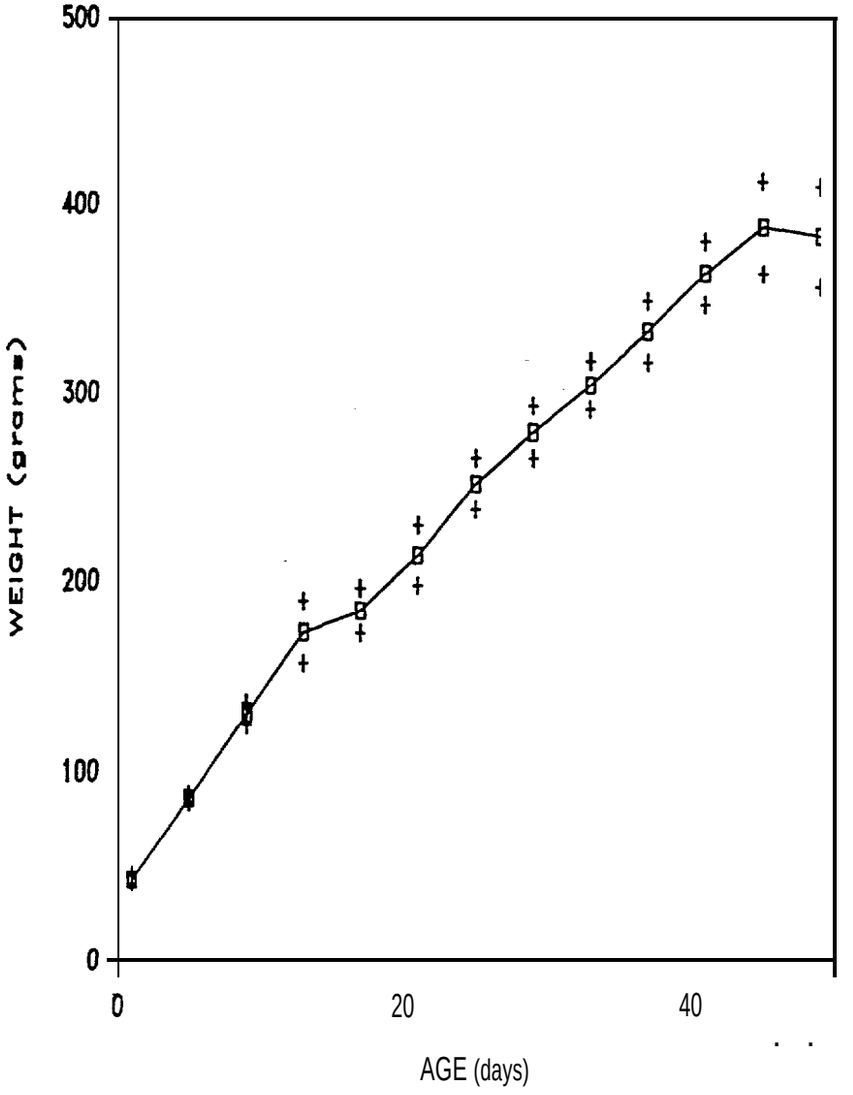


Figure VII-9. Growth rates of Wedge-tailed Shear water chicks from 1983 Non-Experimental birds (n=8) (mean ± SEM).

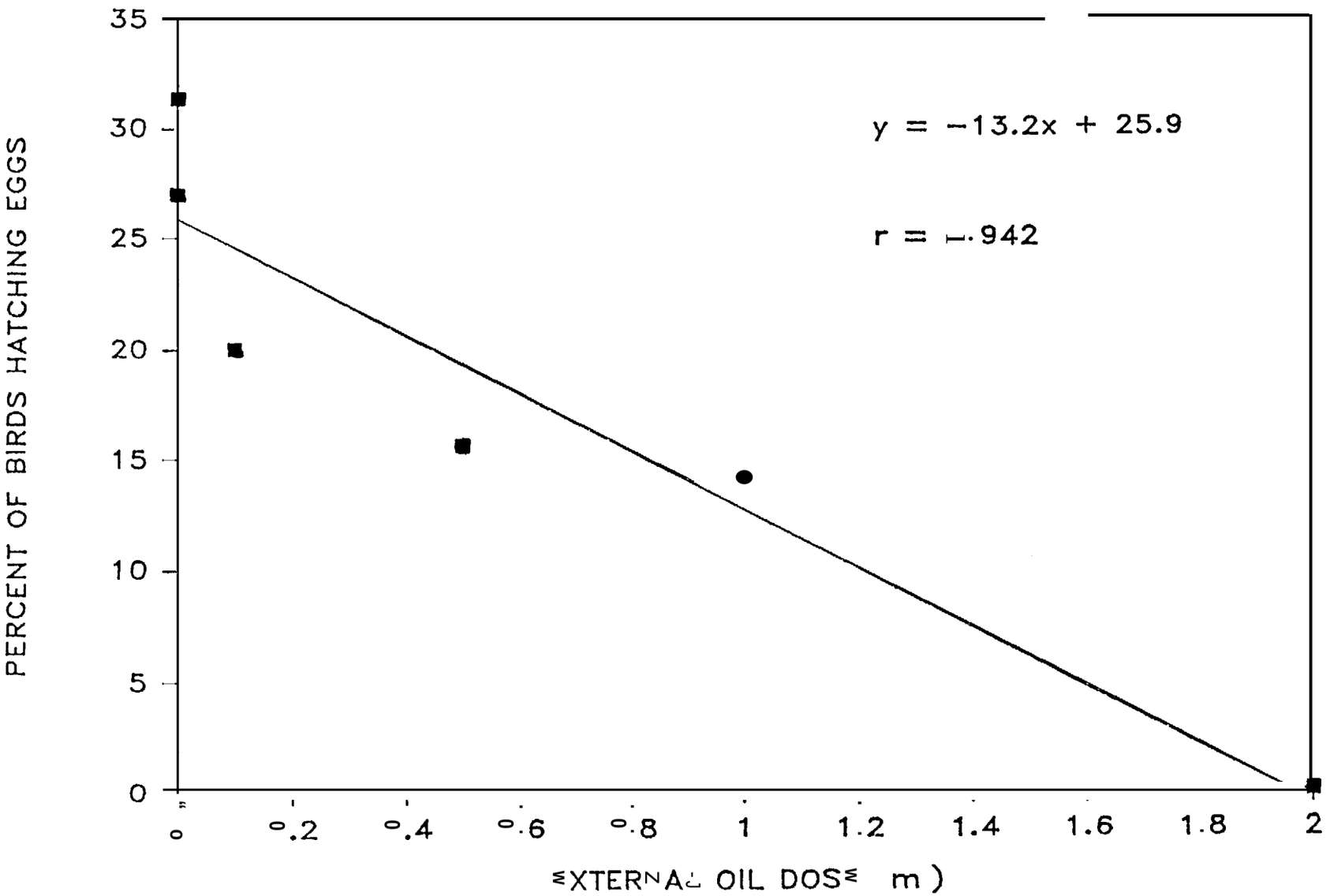


Figure VII-10. Effect of external oil dose on percent of Wedge-tailed Shearwaters hatching eggs in 1983 and 1984.

Table VII-4. Wedge-tailed Shearwater exposure to weathered crude oil: 1984 Dose response to external application.

Dose*	Birds	Birds Returning		Birds Incubating		Hatching Success		Chicks Raised		Net Breeding Success
		#	%[a]	#	%	#	%[b]	#	%	%[c]
0 (Control)	51	31	61	27	53	16	59	12	75	24
0.1 ml External	60	29	48	24	40	12	50	5	42	8[d]
0.5 ml External	64	31	48	21	33[d]	10	48	6	60	9[d]
1.0 ml External	60	27	45	116	27[e]	9	56	3	33[d]	se

*: Chi-squared

a: % Birds Returning is the number returning after exodus in 1984 divided by the number in the treatment group in 1984.

b: % Hatching Success is the number of incubating birds which hatched chicks divided by the total number of birds in the group incubating eggs in 1984.

c: Net Breeding Success is the number of chicks raised in 1984 divided by the total treatment group size.

': Significantly different from control ($p<0.05$).

': Significantly different from control ($p<0.005$).

incubated eggs in 1984. The average distance moved by birds in any group was much less than the plot size; the maximum distance moved was 32 m. If birds were deliberately selecting new burrows away from the site where they were exposed to oil, differences would have been apparent in the distance to new burrows of dosed birds.

Significantly fewer of the 1983 externally oiled birds returned and incubated eggs in the study plot in 1984 (Table VII-5). The net breeding success of both oral (2 ml) and external (2 ml) groups was lower than that of the control group. Externally oiled birds were most affected by the long-term effects of a single exposure to oil. A smaller percentage of birds oiled externally in 1983 returned in 1984, a lower percentage laid eggs, and the net production of chicks was lower than for the other 1983 groups. Many of the 1983-treated birds returning in 1984 had not been observed after they had been treated in 1983. These birds appear to have abandoned the colony that year, but had survived, and returned to breed in 1984.

Birds returning in 1984 and 1985 were tabulated on an individual basis regardless of the treatment history of their mates. This was necessary because many of the returning birds switched mates in 1984 and 1985, making simple analysis difficult, as some birds paired with unhanded birds or with birds from other treatment groups (Table VII-6).

D. Analysis of Reduced Breeding Success: Effect of Mate Fidelity

There are two alternative hypotheses to propose for the second year decline in breeding success of oil-dosed birds. Ingestion of oil resulting in liver hypertrophy and induction of mixed function oxidases could result in long-term modification or impairment of hormonal balance and disruption of courtship and breeding. Long-term damage to liver and kidney or limited recovery from the toxicity could function as physiological stressors, resulting in compensatory pituitary release of corticotropin (ACTH) and adrenal corticosterone release with a suppressive effect on breeding (Peakall et al. 1981; Harvey et al. 1984).

A second possible explanation for the reduced breeding in the year following dosing may involve breeding behavior and the disruption of pair bonds. Several multi-year studies have demonstrated a close association between breeding success and mate fidelity in long-lived seabirds, and that long-term mate fidelity contributes to greater breeding success (Coulson 1966; Kepler 1969; Ollason and Dunnet 1978, 1983). The sequence of oil exposure and breeding failure in the year of dosing may have caused separation of the dosed pairs. Subsequent pairing of the dosed birds with new mates in the following year could have resulted in less successful breeding. Elements of both of these possibilities could be acting together. Oiled birds experienced breeding failure in the year of dosing may have caused mate switching, and the residual toxic effects on individual birds could have compounded the reduced breeding success attributable to the inexperience and reduced teamwork with a new mate.

The records of control and dosed shearwaters were examined for breeding success in the year after dosing, and correlations were made for both mate fidelity and previous breeding success. The mate fidelity of birds exposed to oil in 1983 and returning in 1984 is given in Table VII-7. Thirty-seven percent of all control birds returned with their mates of 1983 while only 20% of the orally dosed and only 7% of the externally dosed birds which returned in 1984 were found with their mates of 1983.

Table VII-5. 1984 Reproductive Success of Wedge-tailed Shearwaters experimentally exposed to oil in 1983.

Group*	Birds Returning		Birds Incubating		Hatching Success		Chicks Raised		Breeding Success as percent of 1983 group[c]
		%[a]		%		%[b]		%	
Control	87	38	68	30	48	71	29	60	13
Oral	46	37	31	25	18	58	12	67	10
External	30	25[d, e]	22	18[d]	13	59	10	77	8

* Chi-squared

[a] % Birds Returning is the number seen in 1984 divided by the total number in the group in 1983.

[b] % Hatching Success is the number of incubating birds which hatched chicks" divided by the total number of birds in the group incubating eggs in 1984.

[c] 1984 Breeding Success is the number of chicks raised in 1984 divided by the total 1983 group size.

[d] Significantly different from control group ($p<0.05$).

[e] Significantly different from oral group ($p<0.05$).

TABLE VII-6. MATING COMBINATIONS OF EXPERIMENTAL WEDGE-TAILED SHEARWATERS. MANANAISLAND, 1985

COMBINATION OF PAIR	NUMBER OF EGGS LAID	
CONTROL X CONTROL	6	
CONTROL X NON-EXPERIMENTAL	26	
CONTROL X DOSED	8	
CONTROL X MATE UNKNOWN	7	
ORAL DOSED X ORAL DOSED	2	
ORAL X CONTROL	3	
ORAL X NON-EXPERIMENTAL	13	
ORAL X OTHER DOSED	0	
ORAL X MATE UNKNOWN	5	
2 ML EXTERNAL X 2 ML EXTERNAL	1	
2 ML EXTERNAL X CONTROL	1	
2 ML EXTERNAL X NON-EXPERIMENTAL	15	
2 ML EXTERNAL X OTHER DOSED	1	(0.1 ML)
2 ML EXTERNAL X MATE UNKNOWN	5	
0.1 ML EXTERNAL X 0.1 ML EXTERNAL	2	
0.1 ML EXTERNAL X CONTROL	0	
0.1 ML EXTERNAL X NON-EXPERIMENTAL	6	
0.1 ML EXTERNAL X OTHER DOSED	1	(2 ML EXTERNAL)
0.1 ML EXTERNAL X MATE UNKNOWN	1	
0.5 ML EXTERNAL X 0.5 ML EXTERNAL	0	
0.5 ML EXTERNAL X CONTROL	1	
0.5 ML EXTERNAL X NON-EXPERIMENTAL	12	
0.5 ML EXTERNAL X OTHER DOSED	1	(1.0 ML EXTERNAL)
0.5 ML EXTERNAL X MATE UNKNOWN	4	
1.0 ML EXTERNAL X 1.0 ML EXTERNAL	1	
1.0 ML EXTERNAL X CONTROL	4	
1.0 ML EXTERNAL X NON-EXPERIMEh'TAL	5	
1.0 ML EXTERNAL X OTHER DOSED	1	(0.5 ML EXTERNAL)
1.0 ML EXTERNAL X MATE UNKNOWN	2	

TABLE VII-7. MATE FIDELITY OF 1983 EXPERIMENTAL SHEARWATERS RETURNING IN 1984.

TREATMENT GROUP	RETURN IN 1984		RETURN WITH 1983 MATE		RETURN WITH NEW MATE		RETURN ALONE		NOT RETURN	
	#/TOTAL	%	#	%	#	%	#	%	#	%
CONTROL	87/231	38	32	37	48	55	7	8	144	62
ORAL (2 ML)	46/127	36	9	20	28	61	9	20	81	64
EXTERNAL (1 ML)	29/119	24	2	7	22	76	5	17	90	76

*: Chi-square analysis
(Ho: Proportions of returning birds are the same for control, oral and external groups.)

Return proportions of control and dosed birds were significantly different ($p<0.005$, $F=19.33$, $df=6$).

More control birds returned.

More control birds returned with 1983 mate.

More dosed birds returned with a new mate.

More dosed birds returned alone.

Significantly fewer birds returned, significantly more dosed birds returned alone, and a significantly greater percentage of dosed birds returned with new mates in 1984.

The 1984 breeding success of birds returning with their mate of 1983 or with a new mate is given in Table VII-8. Significantly more birds which returned with their 1983 mates laid eggs, a greater percentage of those eggs hatched, and a higher percentage of chicks fledged compared to birds breeding with a new mate in 1984 ($p < .02$). This is true for all groups taken together and for each separate treatment group. The numbers of returning birds are small, and most individual comparisons are resultingly not significant. It appears clear that mate fidelity of shearwaters has a positive effect on breeding success and that changing mates results in slightly lower breeding success.

The data were further examined to attempt to discover correlations which might explain the causes of mate switching and decreased mate fidelity after treatment with oil. A positive correlation was found with breeding success after oil dosing in 1983 and mate fidelity in 1984. Table VII-9 gives data for control and treatment of birds which remained together or switched mates, correlated with their breeding success in 1983. Breeding success was separated into four groups: birds which did not lay an egg; birds laying an egg that did not hatch; birds hatching a chick that died early; and birds which successfully raised a chick to 250g. Seventy-six percent of the control birds which did not lay in 1983 *changed mates* in 1984, while 90% of those birds which successfully raised a chick remained with the same mate in 1984. Intermediate success resulted in intermediate values for birds which changed mates. The same relationships hold for orally and externally dosed birds, although the numbers of birds in each category is small. The summary data for all groups is highly significant and shows that 80% of all birds not laying in 1983 changed mates in 1984, while 85% of all birds successfully raising a chick remained together.

These data strongly support the hypothesis that breeding failure in 1983 of dosed birds as a result of oil application resulted in mate switching in 1984, and the data from Table VII-8 demonstrates that mate switching resulted in overall lowered breeding success.

A further examination was conducted to determine whether changing burrows had any effect on established breeding pairs of shearwaters. Table VII-10 presents data from 44 pairs of birds which remained together from 1983 to 1984. Sixty-four percent switched burrows, but the laying frequency, hatching success and fledging success were nearly identical, indicating that there is no deleterious effect of changing breeding burrows if the pair remains together. This was not unexpected, as the tuff soil of the island is delicate and many burrows collapsed each year during the winter rains.

Data from the 1984 oil exposure study were analyzed separately for the same trends. The intermediate oil exposure levels in 1984 resulted in less severe effects on incubation and breeding success (Table VII-4), which is reflected in less severe effects on mate fidelity and breeding success in the year after exposure. 1985 proved to be a less successful year for control and non-experimental birds, further reducing any differences between controls and oil dosed groups (See Table VII-11). Table VII-12 presents the 1985 mate fidelity of birds dosed in 1984. Only 9 birds remained with their mates of 1984 while 50 changed mates. The

TABLE VII-8. EFFECT OF MATE FIDELITY ON 1984 BREEDING SUCCESS IN SHEARWATERS EXPOSED TO OIL.

BIRDS RETURNING WITH THE SAME MATE IN 1984

	CONTROL		ORAL		EXTERNAL		TOTALS	
	#	%	#	%	#	%	#	%
1984 SUCCESS								
BIRDS RETURNING	28		8		3		39	
BIRDS NOT LAYING	5	8	0	0	0	0	5	13
BIRDS LAYING EGGS	23	82	8	00	3	100	34	87
BIRDS HATCHING CHICKS	17	83	6	75	3	100	28	82
BIRDS RAISING CHICKS	12	63	6	00	2	67	20	71
NET BREEDING SUCCESS								51.3

BIRDS RETURNING WITH A NEW MATE IN 1984

	CONTROL		ORAL		EXTERNAL		TOTALS	
	#	%	#	%	#	%	#	%
1984 SUCCESS								
BIRDS RETURNING	48		27		21		96	
BIRDS NOT LAYING	9	19	10	37	4	19	23	24
BIRDS LAYING EGGS	39	81	17	63	7	81	73	76
BIRDS HATCHING CHICKS	26	67	12	71	10	59	48	66
BIRDS RAISING CHICKS	15	58	6	50	8	80	29	60
NET BREEDING SUCCESS								30.2

All birds with same mate have significantly better breeding success than birds with new mates ($p = 0.0195$, Sign test).

TABLE VII-9. INFLUENCE OF 1983 BREEDING SUCCESS ON 1984 MATE FIDELITY IN WEDGE-TAILED SHEARWATERS.

TREATMENT GROUP	CONTROL		ORAL		EXTERNAL		ALL GROUPS	
	SAME[a] MATE 1984	NEW MATE 1984	SAME[c] MATE 1984	NEW MATE 1984	SAME[c] MATE 1984	NEW MATE 1984	SAME[b] MATE 1984	NEW MATE 1984
PREVIOUS BREEDING SUCCESS								
DID NOT LAY IN 1983	8 (24)[1]	26 (76)	4 (19)	17 (81)	3 (5)	18 (86)	15 (20)	61 (80)
LAID, NO HATCH IN 1983	6 (32)	13 (68)	2 (20)	8 (80)	0 (0)	2 (100)	8 (26)	23 (74)
HATCHED, CHICK DIED IN 1983	5 (63)	3 (38)	0 (0)	1 (100)	0 (0)	0 (0)	5 (56)	4 (44)
CHICK RAISED IN 1983	9 (90)	1 (10)	2 (67)	1 (33)	0 (0)	0 (0)	11 (85)	2 (15)
TOTAL NUMBER BIRDS RETURNING IN 1984	28 (39)	43 (61)	8 (23)	27 (77)	3 (13)	20 (87)	39 (30)	90 (70)

*: Chi-squared analysis.

1: Percent of treatment group returning with same mate or new mate.

a: Same mate control birds had significantly better breeding success than new mate control birds ($p<0.001$, $df=3$).

b: For all groups same mate pairs had significantly greater breeding success than new mate pairs ($p<0.001$, $df=3$).

c: Numbers of oral and external birds were too low for statistical analysis.

TABLE VII-10. BREEDING SUCCESS OF ESTABLISHED PAIRS OF WEDGE-TAILED SHEARWATERS
EFFECT OF BURROW SWITCH, 1983-1984

	SAME BURROW		NEW BURROW	
	#	z	#	%
NUMBER BIRDS RETURNING	16		28	
BIRDS LAYING EGGS	14	88	25	89
BIRDS HATCHING CHICKS	12	86	21	84
BIRDS FLEDGING CHICKS	8	67	14	67

TABLE VII-11. 1985 BREEDING SUCCESS OF 1984 OIL EXPOSED SHEARWATERS.

TREATMENT GROUP	BIRDS DOSEI) #	BIRDS RETURNING #	%	BIRDS INCUBATING #	%	HATCHING SUCCESS #	%	CHICKS RAISED #	%	NET BREEDING SUCCESS %
CONTROLS	51	16	31.4	6	37.5	4	66.7	3	75.0	18.8
0.1 ML EXTERNAL	60	14	23.3	12	85.7	9	75.0	2	22.0	14.3
0.5 ML EXTERNAL	64	20	33.3	18	90.0	13	72.2	6	46.2	30.0
1.0 ML EXTERNAL	60	18	30.0	15	83.3	8	53.3	6	75.0	33.3

TABLE VII-12. RETURN OF SHEARWATERS IN 1985 FOLLOWING OIL EXPOSURE IN 1984.

TREATMENT GROUP	RETURN IN 1985		RETURN WITH 1984 MATE		RETURN WITH NEW MATE		RETURN ALONE		NO RETURN IN 1985	
	#/TOTAL	%	#	%	#	%	#	%	#	%
CONTROL	19/51	37.3	4	21.1	12	63.2	3	15.8	32	63
1.0 ML EXTERNAL	18/60	30.0	3	16.7	12	66.7	3	16.7	42	70
0.5 ML EXTERNAL	20/64	28.1	0	0.0	15	83.3	5	27.8	44	69
0.1 ML EXTERNAL	14/60	23.3	2	14.3	11	78.6	1	7.1	46	77

return rate of controls was 37% compared to 30%, 28%, and 23% for 1.0, 0.5, and 0.1 ml dosed groups, but the differences are not significant. The mate fidelity of the returning birds was a very low: 21.1% for controls, and 16.7%, 0%, and 14.3% for the respective dosed groups. As a result, there was no demonstrable effect of mate fidelity on 1985 breeding success. Similarly, data presented in Table VII-13 shows no correlation between breeding success in 1984 and mate fidelity in 1985.

The data were combined for all years for a summary of the influence of breeding success on mate fidelity (Table VII-14). The vast majority of birds which did not lay in one year returned with a new mate in the subsequent year (82%). The fraction of birds returning with their previous mate increased with relative breeding success. Fifty-six percent of all birds which successfully raised a chick returned with their same mates in the next year.

E. Discussion

The striking effects of oil contamination on breeding success of Wedge-tailed Shearwaters were revealed by the significant lowering of egg production and hatching success after only 0.1 ml weathered Santa Barbara crude oil was applied to the breast plumage. Stepwise increases in exposure yielded progressively reduced breeding, with complete breeding failure occurring at 2.0 ml. Birds dosed orally with 2.0 ml oil in gelatin capsules were also adversely affected but to a lesser extent. Externally oiled birds displayed reduced breeding success in the year after treatment, suggesting that long-term breeding depression may result when seabirds are externally exposed to a single small amount of oil.

The response of shearwaters to oil exposure was varied. Many externally oiled birds abandoned, apparently not returning to the colony for one or two breeding seasons. Some orally dosed and externally oiled birds returned to the colony, laid eggs and abandoned them during incubation. Treated birds which returned did not move to other parts of the colony with any greater frequency than controls. The predisposition to return and attempt to breed apparently remained, but the cooperative effort required of both birds to hatch and raise a chick was evidently impaired. Orally dosed birds which returned and successfully hatched chicks were apparently able to forage and feed chicks at the same rate as controls, as the growth rates of surviving chicks were not different from controls. Chicks of orally dosed birds had unexplained but significantly poorer survival than control chicks.

The effects of the oral dose of oil in this study were of longer duration than observed by Trivelpiece et al. (1984) who fed adult Leach's Storm petrels 0.1 ml of Prudhoe Bay crude oil during the early chick rearing stage of the breeding cycle. They found that storm-petrels did not forage for a few days after being dosed with oil; hence, their chicks were severely affected. The adults recovered quickly and were able to successfully raise chicks large enough to survive the 4 or 5 day period of starvation. In this shearwater study, in which oil was applied a few days before egg formation was to begin, many dosed birds abandoned the colony and even those adults which returned to lay and incubate were unsuccessful. Chick survival of orally dosed adult birds was reduced. Oil exposure did not change the length of the pre-laying exodus of those birds which did return but resulted in a majority of oiled birds abandoning the colony. The percentage of oil-exposed birds returning to the colony was lower than for controls. The most likely cause of abandonment was stress-related suppression of breeding.

TABLE VII-13. INFLUENCE OF 1984 BREEDING SUCCESS ON 1985 MATE FIDELITY IN WEDGE-TAILED SHEARWATERS.

	CONTROL		1.0 ML EXT		0.5 ML EXT		0.1 ML EXT	
PREVIOUS SUCCESS	SAME MATE 1985	NEW MATE 1985	SAME MATE 1985	NEW MATE 1985	SAME MATE 1985	NEW MATE 1985	SAME MATE 1985	NEW MATE 1985
DID NOT LAY IN 1984	1 (10)[1]	9(90)	2 (18)	9 (82)	o (o)	10 (loo)	2 (29)	7 (78)
LAID, NOHATCH IN 1984	1 (50)	1 (50)	1 (50)	1 (50)	o(o)	2 (loo)	o (o)	2 (loo)
CHICK HATCHED IN 1984	1 (loo)	o (o)	0 (0)	0 (0)	o(o)	2 (loo)	o (o)	2 (loo)
CHICK RAISED IN 1984	1 (33)	2 (67)	o (o)	2 (loo)	o(o)	1 (loo)	0 (0)	0 (0)
TOTAL NUMBER BIRDS RETURNING IN 1985	4 (25)	12 (75)	3 (20)	12 (80)	o (o)	15 (loo)	2 (15)	11 (85)

[1]: PERCENT OF TREATMENT GROUP RETURNING WITH SAME MATEOR NEW MATE, EXCLUDES BIRDS RETURNING ALONE OR WITH UNKNOWN MATE.

TABLE VII-14. SUMMARY OF THE EFFECTS OF BREEDING SUCCESS ON MATE FIDELITY IN WEDGE-TAILED SHEARWATERS"*.

	SUCCESS IN PREVIOUS YEAR							
	DID NOT LAY		LAID NO HATCH		HATCHED CHICK DIED		CHICK RAISED	
	#	%	#	%	#	%	#	%
RETURN WITH OLD MATE	17	(18)	14	(22)	11	(31)	31	(56)
RETURN WITH NEW MATE	79	(82)	49	(78)	25	(69)	24	(44)
TOTALS :	96		63		36		55	

*: Chi square analysis ($p<0.001$, $F=27.28$, $df=3$).

Significantly more birds return with new mates when they fail to lay an egg, hatch an egg or fledge a chick.

Shearwaters were banded and dosed during the courtship phase of the breeding season, before the pre-laying exodus and egg formation. It was observed during 1983 and 1984 that birds were away from the colony for a pre-laying exodus of 24-29 days with no differences between control and oil treated groups. All groups left the island for a slightly longer period in the year of banding and dosing than in the second year, but only the exodus lengths of the control group were statistically different between years. The length of exodus found in this study was longer than observed by Shallenberger (1973), but the difference may be because, in this case, the colony was checked only during the morning while he also observed birds in the colony at night when they are more likely to be on the island. It is possible that banding and dosing may have caused a premature exodus from the island, but any effect of dosing with oil appeared not to be additive to the handling and banding disturbance. These observations are different from those of Roberts et al. (1974) who found in Australia that females began rapid egg yolk growth before their exodus. It is not known whether oil dosing had a direct effect on egg formation as demonstrated with Cassin's Auklets in this study and by Ainley et al. (1981). It is unlikely that there was an effect, however, as no delay in egg laying was observed.

Some of the reduced survival of embryos and chicks may be attributed to toxicity of oil residues incorporated into eggs during yolk formation. The Santa Barbara weathered crude used in this study has a high proportion of aromatics and could have been embryotoxic if incorporated into yolk during egg formation. (see Hoffman and Gay (1981) for a summary of studies of embryotoxicity of oil components).

In addition to the effects of oil during the year of dosing, shearwaters showed an adverse effect of oil with reduced breeding in the following year. Many oil-treated birds did not return and those that did had reduced breeding success. Birds oiled externally with 2 ml laid fewer eggs and hatched a lower percentage of chicks.

Are Wedge-tailed shearwaters more susceptible to oil than other species of birds? The data suggest that small quantities of oil cause considerable breeding failure and abandonment from the colony, but this may have occurred because shearwaters are less committed to breeding in any given year than some other avian species. Shearwaters are long-lived birds with deferred maturity and low clutch size, reflecting a conservative breeding pattern. Breeding attempts may be suspended in poor years or at times when individual survival is imperiled. The loss of a single season may represent only four or five percent of the expected breeding life of the bird and, if insulted by an oil spill, the individual might forego one season's breeding rather than risk survival while attempting *to* breed.

VIII . CONCLUSIONS

This study has investigated both short and long-term effects of single small exposures of weathered crude oil on two species representing very different taxonomic groups of seabirds: Cassins' Auklets, a northern latitude alcid specialized for diving; and Wedge-tailed shearwaters, a tropical procellariform specialized for long flights in search of surface prey which are seized on the wing or captured in shallow plunge-dives. Acute toxicity studies of both crude and refined residual fuel oils (Bunker C) were additionally conducted with a second alcid species, the Common Murre.

A. Acute Toxicity of Petroleum

The acute toxic effects of exposure to Santa Barbara weathered crude oil, demonstrated in this study, included dissociation of liver cells, focal necrosis of collecting tubules in the kidney, and Heinz-body hemolytic anemia. Auklets exposed externally, under captive conditions, *to* 3 or 5 ml of. weathered crude oil died within four days. The cause of death appeared *to* be a combination of stress from oil and captivity complicated by anemia and impaired liver and kidney function.

The acute toxicity of petroleum is undoubtedly dose dependent and is related both to the extent of plumage fouling and waterlogging as well as to the amount of oil ingested during preening in efforts to clean oil from feathers. Many seabirds exposed to viscous oils from spills become fouled and flightless and must swim to land to avoid drowning. Birds which are lightly oiled may clean themselves and survive. Acute captive trials demonstrated that exposure to 3 ml of oil spread evenly on the wings or breast of Cassin's Auklet caused severely matted plumage and was a lethal dose.

B. Long-Term Field Studies

Exposure of auklets to 1 ml oil orally by gelatin capsule had no effect on breeding success and only minor effects on the morphology of blood cells and a short delay in egg laying. Exposure to a 1 ml dose by application to the breast plumage, however, had significant deleterious effects egg laying breeding success, and mate fidelity in a subsequent year. External exposure of oil to shearwaters caused increased abandonment before laying and breeding failure with a dose-dependent response.

Other investigators have demonstrated similar effects on reproduction with timing of the oil dose being an important variable. Exposure to crude oil prior to the breeding season delayed sexual maturation or onset of lay in captive mallards (Holmes et al. 1978; Coon and Dieter, 1981). Dosing with oil at the time of egg formation resulted in a delay in initiation of yolk formation in auklets (Ainley et al. 1981), and Engel et al. (1978) and Wooton et al. (1979) found an interruption in yolk synthesis in Japanese Quail after exposure to PBCO or Bunker C (No. 6 fuel oil). Some crude oils are embryotoxic and if ingested during the period of egg formation may affect the development of progeny (Vangilder and Peterle, 1980; Gorsline and Holmes, 1982b). Trivelpiece et al. (1984) showed that at later stages of the breeding season, exposure of adult Leach's Storm-petrels (Oceanodroma leuchorhoa) to PBCO resulted in chick loss because parent birds did not forage and feed their young. Additionally, oil ingested incidentally by adults and transferred to nestlings may be harmful, as single doses of Prudhoe Bay crude, South Louisiana crude, or No. 2 fuel oil fed to nestling gulls, guillemots, or ducklings depressed

growth and altered behavior (Szaro and Albers, 1978; Szaro et al. 1981; Miller et al. 1978; Peakall et al. 1980, 1983).

In this Study, it was not possible to determine physiological factors responsible for the observed decreased breeding success. In most cases, birds abandoned their nests for one or more seasons and no physiological measurements were possible. Many auklets dosed during incubation, however, did remain and complete incubation but with reduced hatching success. Transfer of oil to eggs and direct embryo toxicity may have been a factor in reduced matchability, but reduced incubation attentiveness as a consequence of partial abandonment may also have contributed to reduced matchability.

The most striking effects of oil observed in field studies were the combination of abandonment and decreased mate fidelity by both auklets and shearwaters. Birds which abandoned before egg laying failed to return to breed in subsequent years or returned with different mates. Birds which remained throughout the breeding season after being dosed had reduced breeding success and most returned with a different mate in the following year.

The consequence of changing mates was a trend toward reduced breeding success in the year after oil exposure. Auklets and shearwaters are long-lived seabirds with prolonged breeding seasons in which a coordinated effort on the part of both adults is necessary for successful reproduction. A substantial amount of learning is probably associated with breeding success, as older birds are usually more successful, and maintenance of the pair bond improves breeding success in most species of seabirds (Coulson 1966, Mills 1973, Ollason and Dunnett 1978). This was demonstrated by auklets breeding in NOAA boxes. The "NOAA" treatment group as a whole was an established breeding population with many birds having bred in those boxes in previous years. Exposure to oil during incubation caused breeding failure, but many pairs remained together and laid a second egg. The net breeding success of this group was higher than new pairs nesting in MMS boxes, both as a result of less abandonment of the first egg and because of a much higher relaying frequency. Pairs which remained together in the year after dosing demonstrated the same trend, with a higher relaying frequency after the failure of their first egg in 1985. Most oil-exposed auklets and shearwaters, however, changed mates after breeding failure, and controls which failed also changed mates.

There is no information on the previous breeding history of these shearwaters, although many of the birds could have bred in the same area of the Manana colony in previous years. The low return frequency for all birds and the low mate fidelity of control birds was unexpected, as established breeding pairs of shearwaters and other procellariids are reported to have high mate fidelity (Shallenberger, 1973; Ollason and Dunnett, 1978). Positive correlations were found for mate fidelity and breeding success, and for mate switching and low breeding success. This is different from Northern Fulmars which remain together as pairs independent of breeding success (Ollason and Dunnett, 1978, 1983). Mate switching had a negative effect on breeding success in shearwaters, although there may be some selective advantage in changing mates after breeding failure, if the failure is due to lack of coordinated breeding activity or other incompatibility.

The overall effect of oil exposure is difficult to analyze in a field study because of disturbance at the breeding site. All dosing and subsequent observations were conducted at the breeding burrow with the possible consequence of disrupting breeding independent of oil treatment. Some mate switching of auklets, for example, may have occurred because females left the area while males remained at the site. If the females change sites because of the association with disturbance and oil, this study would be compounding the negative effects of oil compared to the situation of an auklet encountering oil at sea and experiencing breeding failure. These studies were conducted with appropriate controls, but the possibility of a synergistic effect of observer disturbance compounded by oil exposure cannot be eliminated. The comparison study of NOAA and MMS boxes probably comes close to addressing this problem, as both sexes of auklets were committed to NOAA boxes and there was no sex difference in return *to* the colony in the year following oil dosing.

The impacts of an oil spill on an established colony may be similar to the net results of our study. Many birds would be killed and many pairs would be disrupted if single members were oiled. Disruption of established breeding pairs and replacement by inexperienced or marginal breeders would result in a depression in net breeding success of the colony which could last for more than one year. The recovery of the colony would be slower than the normal reproductive potential of the colony because of the reduced success of newly formed pairs.

This study is one of the only two reports which provide information on the long-term effects of oil on seabirds. The study by Morant et al (1981) documented the success of rehabilitation of oiled penguins in South Africa, and while not experimentally controlled, presents some pertinent findings. During the period 1970-1979, more than 2,600 oiled penguins were cleaned, rehabilitated, and released after tanker accidents. Thirty percent of the rehabilitated birds were subsequently observed returning to breeding colonies, but only 20% of the cleaned penguins returning to the colonies were observed incubating eggs or raising chicks. Only six percent of all rehabilitated birds subsequently bred, suggesting that most of the oiled birds did not recover completely. A more detailed study would be needed to determine whether the oiled penguins were affected for more than one season.

The damaging effects of oil on seabird populations have been the subject of speculation (Ainley and Lewis 1974), but no data other than this study of multi-year effects on breeding have been published. The present study suggests that the effects of a large oil spill could be more severe than merely disrupting reproduction when birds are exposed to oil during the breeding season. If some long-term effects are a consequence of prolonged impairment of physiological responses, exposure to oil during the non-breeding season could easily affect later breeding success. If the long-term depression in breeding reflects mate switching in response to adverse conditions only during the breeding season, the consequences of an oil spill in the non-breeding season could be less severe, but the reduced success of newly paired birds in a subsequent year would still result in lowered net breeding success.

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APPENDIX A

Gross Necropsies and Histopathology Reports of the Six Captive Auklets

VETERINARY MEDICAL TEACHING HOSPITAL
UNIVERSITY OF CALIFORNIA, DAVIS

PATHOLOGY REPORT

CLINIC NO. _____ PATHOLOGY NO. 82R 470

SPECIES avian SEX _____ AGE adult DATE 1/28/82

BREED Cassin'sauklet COLOR red PATHOLOGIST Dr. Lowenstine/Gillett

TAG NO. OR NAME _____ STUDENT _____

CLINICIAN _____ STUDENT _____

OWNER Dr. M. Fry ADDRESS Avian Science Dept, UCD

DOCTOR _____ ADDRESS _____

SPECIMEN carcass DIED OR KILLED D HRS. 3 DESTROYED BY _____ PRESERVATIVE none

Postmortem STATE mod.autolysis **NUTRITIONAL STATE** fair REPORTED 5/4/82

PATHOLOGIC DIAGNOSIS:

1) TOXICOSIS, EXPERIMENTAL, CRUDE OIL.
2) HEPATOCELLULAR DISASSOCIATION WITH HEMOSIDEROSIS OF HEPATOCYTES AND KUPFFER CELLS.
3) VENTRICULITIS, EROSIVE, TRANSMURAL, MODERATE.
4) MULTIFOCAL EROSIVE ESOPHAGITIS AND INGLUVITIS, MILD.
5) TREMADODIASIS, LUNG (TYPHLOCOELOM SP?).,
6) PNEUMOCONIOSIS, MILD.
7) MILD, MULTI FOCAL, RENAL TUBULAR NECROSIS.

HISTORY:

This female Cassin's auklet was captured with a mist net on the S.E. Farrallon Islands on 1/22/82. Weathered crude oil was applied to her feathers on 1/?/82. She died the morning of 1/28/82 and was examined about 3 hours after her death.

GROSS DESCRIPTION:

Integument: The plumage is covered with oil especially on the breast. The oil does not seem to have penetrated into the downy layer or onto the skin. The uropygeal gland is well developed.

Peritoneum: The afr sacs are slightly opaque (cultured) as if coated by a milky substance (aspirated ingests?).

Digestive Canal: The crop contains one minnow and part of one smelt. A few erosions with thickened plaque-like edges (<1 cm. diam) which are both oval and linear are found in the crop and distal esophagus. The gizzard contains oily black material and fish otoliths. The koilin peels easily from the mucosa. The duodenum contains black-green ingesta but the serosa is not discolored. Segmental darkening of the intestine is seen. The posterior portion is greatly distended by black-green material.

Liver: The liver is of expected size. The gallbladder is distended. There is bile imbibition on the posterior surface of the liver.

Genital System: Testes are inactive {0.4 x 0.15 cm).

Pleura: Thoracic air sacs are transparent.

Respiratory System: Blood is present in trachea and lungs due to method of euthanasia.

Cardiovascular System: NGL.

Lymphoid System: No bursal or thymic remnants are seen. The cecal tonsils just anterior to the cloaca are about 0.25 x 0.1 cm.

Musculoskeletal System: NGL; the pectoral muscles are slightly atrophied.

Nervous System: Brain,.damaged by prosector.

Other Endocrine: The adrenals are very small.

Bone Narrow: NGL.

Special Senses: NE (Not examined).

HISTOPATH SUMMARY:

The following tissues are examined and are considered to be essentially normal: Preen gland, kidney, and pancreas T-2; Heart T-3; Spleen, thyroid & parathyroid, salt gland, testicle and adrenal T-4; Intestines T-5.

There is gold-grown pigment in some of the neurons of the brainstem and optic tectum; other neurons have clumped chromatin (tigoid striping). There is no overt neuronal necrosis.

Golden crystalline pigment is present in nodules of macrophages adjacent to bronchi and parabronchi in the lungs. Bronchiolar glands are dilated by hyalin material and surrounded by fibrous tissue. Lymphoid cells are present focally. The lung is severely congested and blood is present in some airways (agonal). Pigment is present in hypertrophied Kuppfer cells throughout the liver and to a lesser extent in the hepatocytes where it occurs as a faint dusting of green-brown granules. Pigment is present in macrophages in airsacs.

The most impressive lesions are in the gastrointestinal tract. There is patchy ballooning of superficial epitheliums in crop and esophagus. A plaque of necrotic keratin containing bacterial colonies is present in the crop mucosa. Intraepithelial vesicles are present containing heterophils and there is extensive heterophilic infiltration in the edematous submucosa beneath the mucosal ulcers and erosions. In the ventriculus a mild heterophilic infiltrate is seen within the mucosa beneath focal absence of koilen. Deep within this in the submucosa is a focus of edema and heavier heterophilic infiltrate around some necrotic material. A few giant cells are also seen.

Lymphoid tissue in all loops of bowel in varying amounts. Interstitial cells of

necrosis and cellular debris in both the crop and esophagus. Glands in the proventriculus are distended by granular proteinaceous material and cytoplasmic debris. Lymphoid cells are present in the lamina propria, mucosa and in the serosa. There is widespread separatio of the korlin layer from the mucosa. There is focal hypereosinophilia of this layer representing korlin necrosis. Red cell nuclei are present reflecting hemorrhage. A few subjacent glandular lumina are dilated by cellular debris and bacteria (cocci). Similar bacteria are in the superficial koilin. The muscularis in this area contains vessels cuffed by mixed inflammatory cells mainly heterophils and the serosa is thickneed and edematous. The duodenal mucosa is autolyzed and numerous bacteria are present in the lumen and in crypts. There is modest infiltrate of lymphocytes and plasma cells in the lamina propria. There is a mild accumulation of orange pigment in the proximal intestinal lumen (blood pigments?) which becomes more marked in the distal loops. There is mild necrosis of lymphoid tissue within the cecal tonsils.

MICROBIOLOGY:

Both hemolytic and non-hemolytic E. coli were cultured in moderate numbers from a swab of the air sacs.

COMMENT:

The lesion attributable to captivity and/or toxicosis are those in the liver and in the kidney. Lesions in the upper GI tract were seen in both control and oiled birds, and were often more chronic than the duration of captivity. Hepatocellular and kupffer cell iron increase in response to increased delivery of iron to the liver, whether from hemolysis or from increased absorption through the GI tract. Toxicosis, especially lead poison, have been shown to cause hepatocellular hemosiderosis. Reports of crude oil exposure In peking ducks and Herring gulls (Miller et al in Animals as Monitors of Environmental Pollution, pp. 27-40) reported hepatocellular hypertrophy not pigmentation. Other lesions reported in gulls and ducks were vacuolation of enterocytes. Unfortunately mucosal preservation in oiled birds which died was inadequate to make this evaluation. Salt gland hypertrophy was seen in gulls given 100Z salt water to drink and exposed to crude oil by gavage. This change was apparent 7 to 10 days after exposure. No morphologic change was appreciate. in the auklets' salt glands but they died or were sacrificed before 7 days post exposure. Adrenal cortical hyperplasia is another lesion reported in oiled birds. The captive birds in this study had large (subjective) cortical cells than the bird which died shortly after capture, but adrenals were not grossly enlarged.

Linda J. Lowenstine, Veterinary Pathologist

VETERINARY MEDICAL TEACHING HOSPITAL
UNIVERSITY OF CALIFORNIA, DAVIS

PATHOLOGY REPORT

CLINIC NO. - - - - **PATHOLOGY NO,** 82R 473

SPECIES avian **SEX** .—E—. **AGE** immat? **DATE** 1/28/82

BREED Cassin's auklet **COLOR** blue PATHOLOGIST Drs. Lowenstine/Gillette

TAG NO. OR NAME **STUDENT**

CLINICIAN **STUDENT**

OWNER Dr M. Fry **ADDRESS** Avian Science Dept, UCD

DOCTOR. **ADDRESS**

SPECIMEN carcass **DIED OR KILLED** K **HRS.** <1 **DESTROYED BY** exsanguin. **PRESERVATIVE**

POST MORTEM STATE fresh **NUTRITIONAL STATE** **REPORTED** 5/4/82

PATHOLOGIC DIAGNOSIS:

1) TOXICOSIS, EXPERIMENTAL, CRUDE OIL.
2) HEPATOCELLULAR AND KUPFFER CELL HEMOSIDEROSIS, WITH MILD HEPATOCELLULAR DISASSOCIATION.
3) RENAL TUBULAR NECROSIS, LOWER NEPHRONS.
4) MILD, SUBACUTE GASTROENTERITIS, LYMPHOPLASMACYTIC AND GRANULOMATOUS
5) MILD PNEUMOCONIOSIS.

HISTORY:

Captured 1/22/82. Oil put on feathers. (1/25/82) Hct at capture = 60%. On 1/28/92 here Hct was 21%. Other lab data on 1/28/82: WBC = ?; 90% hetero; 8% lymphs; 2% basophils; SGOT = 844; LDH = 536; T.P. = 1.5 gm; Creatinine = 0.8; Uric acid = 12.4; Na+ = 159; K+ = 7.3; Ca = 6.5; Glucose = 310.

GROSS DESCRIPTION:

Integument: Oil coated the outer approx. 1/2 of the feather layer. It did not appear to each the skin.

peritoneum: NSF (No Significant Findings).

Digestive Canal: The crop, gizzard and intestine contained black amorphous material compatible with ingested oil. The koilin layer of the gizzard could easily be pulled from the mucosal surface.

Liver: NSF.

Pancreas: NSF.

Spleen: NSF.

Urinary System: NSF.

Genital System: The ovary contained uniform follicles, all less than 1 mm. in diam. The oviduct was a str-sight, flat structure (not convoluted) approx. 1 mm. wide.

Pleura: NSF.

band approx. 0.3 cm. wide.

Pleura: Air sacs are slightly translucent rather than transparent with occasional more opaque white foci approx. 0.1 cm.

Respiratory System: **Lungs** are light salmon-pink (NGL).

Cardiovascular System: NGL.

Lymph: No bursal nor thymic remnants seen. "Cecal tonsils" are just proximal to the cloaca and are 0.4 x 0.1 x 0.1 cm.

Musculoskeletal System: There is wasting of pectoral muscles but the leg muscles are quite plump still. All muscles are dark red-brown.

Nervous **System:** NGL. .

other Endocrine: The left thyroid is larger than the right (0.4 x 0.3 x 0.2 cm, and 0.2 x 0.2 x 0.2 cm). The adrenals are 0.5 x 0.5 x 0.3 cm.

Bone **Marrow:** The marrow is pale-pink.

Special Senses: NGL.

HISTOPATH SUMMARY:

The following tissues are examined and are considered to be essentially normal: **Brain** and pancreas T-1; Salt gland and uropygeal gland T-4; Adrenal and heart T-5.

There are aggregates of pigment laden macrophages adjacent to bronchi. These are largest adjacent to secondary bronchi but are present alsoin atrial septa of parabronchi. There is fragmentation of smooth muscle-and connective tissue around some bronchi with multi focal **mineralization and** rare multinucleated giant cells. Bronchial glands are dilated by hyalin pink material.

Within the **kidney, scattered tubules have granular cytoplasm and** pyknotic **nuclei** (autolysis? **necrosis). Hepatocytes are individualized and are dusted by green-brown** granules. **Similar** material **is in hypertrophic Kupffer** cells. Scattered lymphocytes and plasma cells are present in low numbers within sinusoids and adjacent to portal and central veins.

In the spleen, the cells of the reticular sheaths are swollen and vacuolated. The lymphoid areas are well populated and a few plasma cells are present. Moderate numbers of lymphocytes and plasma cells are found in the lamina propria of the intestine and in the ureteral wall. In addition, focal accumulation of heterophils is present in the duodenum and to a lesser extent in the cecal tonsils. Many **segments of bowel are** autolyzed beyond interpretation. Lymphocytes and plasma cells are present in the lamina

beneath the ureteral transitional epitheliums. Occasional scattered fibers in the pectoral muscles are atrophic.

COMMENT:

Changes in the gastrointestinal tract are of several weeks duration. Pigmentation is moderate but hepatocellular disassociation is very mild.

See 82R 470 for further comments.

Linda J. Lowenstine, Veterinary Pathologist

/bs

VETERINARY MEDICAL TEACHING HOSPITAL
UNIVERSITY OF CALIFORNIA, DAVIS

PATHOLOGY REPORT

CLINIC NO. _____ **PATHOLOGY NO.** 82R 474

SPECIES avian SEX ___ M A G E DATE 1/28/82

BREED Cassin's auklet COLOR orange PATHOLOGIST Drs. Lowenstine/Gillette

TAG NO. OR NAME _____ STUDENT _____

CLINICIAN _____ **STUDENT** _____

OWNER Dr. M. Fry. ADDRESS Avian Science Dept, UCD

DOCTOR _____ ADDRESS _____

SPECIMEN carcass DIED OR KILLED K HRS. <1 DESTROYED BY exsang. **PRESERVATIVE** _____

POST MORTEM STATE _____ **NUTRITIONAL STATE** _____ **REPORTED** 5/4/82

PATHOLOGIC DIAGNOSIS:

1) TOXICOSIS, EXPERIMENTAL, CRUDE OIL.
2) HEPATOCELLULAR AND KUPFFER CELL HEMOSIDEROSIS, MARKED, WITH VERY **MILD HEPATOCELLULAR DISASSOCIATION.**
3) PNEUMOCONIOSIS, **MILD.**
4) MULTIFOCAL **CHRONIC** AND HETEROPHILIC ESOPHAGITIS, PROVENTRICULITIS AND ENTERITIS; ETIOLOGY? PARASITIC.
5) TREMATODIASIS, GASTRIC, POSSIBLY RIBEIROIA SP.

HISTORY:

Control bird captured 1/22/82. Hct ~~at capture~~ 1/25/82 66% to 36% on 1/28. Other lab data on 1/28: T.P. = 2.0; WBC = 5,7000; SGOT = 1180; LDH = 1526; Na = 158; K+ 3.9; Glu = 128; Ca = 6.5; U.A. = 24.9; Creatinine = 0.8; Cortisol = <0.5 mg/ml.

GROSS DESCRIPTION{:

peritoneum: Air sacs are faintly cloudy. There is no abdominal fat.

Digestive Canal: The gizzard contains black oily material. The koilin peels from the mucosa with slight difficulty. The duodenal contents are dark-brown, nearly black, as are the contents of the distal bowel. The mid-portions of the bowel are discolored dark-green and are somewhat dilated. The cloaca and distal bowel are quite dilated.

Liver: The liver is medium-brown and of expected size.

Pancreas: The pancreas is pale and domed but is flatter than that in some of the other birds.

Spleen: The spleen is pale, 0.7 x 0.3 x 0.3 cm.

Urinary System: NGL (No Gross Lesions).

Genital System: The testes are inactive, 0.5 x 0.2 x 0.2 cm.

Pleura: Faintly cloudy air sacs.

Respiratory System: NGL.

Cardiovascular System: NGL.

VETERINARY MEDICAL TEACHING HOSPITAL
UNIVERSITY OF CALIFORNIA, DAVIS

PATHOLOGY REPORT

CLINIC NO. [illegible] PATHOLOGY NO. 82R 556
SPECIES avian SEX M AGE
DATE 1/22/82
BREED Cassin's auklet COLOR
PATHOLOGIST Dr. Lowenstine
TAG NO. OR NAME non STUDENT
CLINICIAN STUDENT
OWNER Dr. M. Fry ADDRESS Avian Science Dept, UCD
DOCTOR ADDRESS
SPECIMEN carcass DIED OR KILLED DHRS. DESTROYED BY PRESERVATIVE formalin
POSTMORTEM STATE NUTRITIONAL STATE REPORTED 5/4/82

PATHOLOGIC DIAGNOSIS:

1) PNEUMOCONIOSIS, MILD TO MODERATE.
2) LYMPHOPLASMACYTIC ENTERITIS, DUODENUM, MILD.
3) INTERSTITIAL CELL HYPERPLASIA, TESTICLES (EARLY PHYSIOLOGIC, RECRUDESCENCE?).
4) GENERALIZED CONGESTION, SHOCK (?).

HISTORY:

This adult, male Cassin's auklet was trapped by mist net on the SE Farrallon Island on 2/22/82 at about 6 am. He died in transit to UCD. Postmortem was conducted at 8:30 am. There were no reported gross abnormalities, Tissues were preserved in 10% neutral buffered formalin.

GROSS DESCRIPTION:

The tissues submitted included: ventriculus, a segment of intestine: a piece of liver; a piece of kidney; testes and adrenals and the apex of the heart and a piece of muscle (pectoral) and pancreas.

The korlin layer of the gizzard is partially detached from the mucosa and is medium green in the fixed state. The loop of intestine submitted has a tan serosa and creamy ingests. There is a faint hepatic reticular pattern, one margin of the piece is deeply stained by bile imbibition. There is a prominent reticular pattern in the surface of the piece of kidney submitted. The testes measure 0.6 x 0.3 x 0.2 cm. and the adrenals 0.6 x 0.4 x 0.2 cm. (each). No lesions are seen in the haert, pectoral muscle or pancreas. The pancreas is cream colored and plump.

HISTOPATH SUMMARY:

There is severe congestion of liver, kidney, lung, pectoral muscle and adrenals and mild autolysis of all tissues examined. Inspite of autolysis there is no disassociation

APPENDIX B
Summary of the Research on Effects of Oils on Eggs and Embryos

SUMMARY OF THE RESEARCH ON EFFECTS OF OILS ON EGGS AND EMBRYOS

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The following tables summarize the literature on the effects of petroleum products on avian eggs and the development of embryos. The work is organized by oil type, with four sections: 1) crude oils, both fresh and weathered; 2) refined products; 3) mixtures of known components of petroleum products; and 4) heavy metal contaminants in oils.

Most of the research has been conducted by the U.S. Fish and Wildlife Service at the Patuxent Wildlife Research Center, Laurel, Maryland, and by the Canadian Wildlife Service, but others have made useful contributions.

The tables are organized with the complete reference on the left and a tabular summary of the research on the right. When comparative studies have been performed on both crude and refined products, the results have been separated into the appropriate sections.

In general, the research supports the conclusion that products containing polyaromatic hydrocarbons (PAH) are the most toxic, but that lower molecular weight solvents must be present to keep the PAH in solution and provide a vehicle for their passage through the egg shell. Therefore, light fuel oils (No.2 fuel oil) refined from asphaltene (naphthenic) based crudes are likely to be the most toxic, followed by crudes with a high naphthenic content (South Louisiana, Kuwait, Prudhoe Bay, and some crudes from Santa Barbara); and bunkers from these stocks. Paraffin-based crudes and light products refined from them appear to be toxic only when the egg is so heavily covered that pores for gas exchange are blocked.

The risk of oil to auklets being monitored for this study is of prime concern because of the continued leakage from the tanker "Puerto Rican". The oil from the sunken stern section of the tanker "Puerto Rican" is a bunker with a high PAH content and few volatile solvents. It appears to be liquid enough to penetrate egg shells and would be quite toxic to eggs. Weathering of this oil could reduce its toxicity somewhat, although most studies show that the toxicity of oils is reduced only after weathering for more that two weeks. It is likely that most birds encountering oil from the "Puerto Rican" will be impacted by oil before it has weathered on the ocean surface for two weeks. Oil which remains in the hull will not weather to any great extent, as most rapid weathering occurs as volatile solvents are lost from a thin film of oil on the water surface. Some small molecular weight water soluble components are lost during weathering, and oxygen and sunlight cause polymerization and photodecomposition of other components.

This summary covers only the literature on effects of oil on eggs and corollary studies of oiled birds transferring oil to eggs. Many other studies have been performed on the effects of oil on reproduction in birds, growth of

chicks, behavioral changes, and toxicity of oil to whole animals and isolated tissues. For a short review of the effects of oil on birds in general, the reader is referred to the recent paper:

> Albers, Peter H. (1983). Effects of Oil on Avian Reproduction: A Review and Discussion. In "The Effects of Oil on Birds : Physiological Research, Clinical Applications & Rehabilitation ." A Multi-discipline Symposium. 1982 Proceedings, Tri-State Bird Rescue & Research, Inc., Wilmington, Delaware, pp. 78-97.

Alber's review briefly covers the scope of the oil problem and contains a good table covering all studies through 1982 on the effects of oil administered to adult or young birds.

SUMMARY OF CRUDE OIL STUDIES:

The toxicity of crudes which have been studied falls in this order:

Most toxic:	South Louisiana
	Kuwait
	Prudhoe Bay
Least toxic:	Libya

Weathering more than ten days lowered the toxicity of Prudhoe Bay crude, but did not clearly alter the toxicity of Libyan crude (which was low to begin with) .

Crudes with high content of polyaromatic hydrocarbons are the most toxic. The initial pumping of South Louisiana crude which was supplied by the American Petroleum Institute (API) was very toxic, more toxic than samples of South Louisiana crude pumped in 1978 and supplied to researchers by the API. Kuwait, Prudhoe Bay, Venezuela, and some California crudes also have significant PAH content, and are toxic to birds and eggs.

The toxicity of crudes is not limited just to killing embryos. South Louisiana crude caused extensive embryo abnormalities at low doses which did not kill the embryos. Doses as low as 5 ul of an oil with high PAH content will cause significant embryo malformations.

Significant toxicity occurs when adult birds encounter oil on water and transfer the oil to the surface of the eggs during incubation. The greatest variables are the type of oil, the age of the embryo at time of oiling, and the extent of the egg shell covered with oil by the adult while turning the eggs. Even non-toxic oils applied to birds caused egg mortality when evenly coated on the eggs.

SUMMARY OF STUDIES OF REFINED PRODUCTS:

Refined products include both distillates and residual oils, making this a very heterogeneous group of products.

The toxicity of refined products falls in this order:

Most toxic: Number 2 fuel oil (mixed stock)
Waste crankcase oil
Bunker C
Lube oil = Clean crankcase oil

Least toxic: Aviation kerosine (prob. virgin stock)

The toxicity of refined products is the result of a combination of toxic components and a solvent vehicle to convey the PAH (poly-aromatic hydrocarbons) through the egg shell. Bunkers contain much higher concentrations of toxic PAH than No.2 fuel oils, but the lower viscosity and greater solvent properties of the fuel oil allow more of the large aromatic components to penetrate the egg shell.

Waste crankcase oil is very toxic because of accumulation of heavy metals, aromatics, high-temperature decomposition products, and dissolved sludges removed from engines. The increased toxicity is clearly demonstrated when waste and clean crankcase oils are compared.

The least toxic refined products are gasolines, kerosines and light lube oils refined from paraffinic crudes (crudes from Pennsylvania and parts of the Middle East). These oils contain almost no PAH and few small aromatic components. Light oils and motor fuels distilled from naphthenic crudes contain a higher percentage of aromatics, but high molecular weight polyaromatics are found in the higher temperature fractions: middle distillates (No. 2 fuel oil), wide cut gas oils (lube oils), and the residium (bunkers, etc). The larger PAH's become concentrated in residual oils such as bunkers.

The composition of fuel and lubricating oils largely depends upon the origin of the crude from which it is distilled, and upon the catalytic cracking processes used to make shorter chain, more volatile products from the higher boiling fractions. In order to obtain gasoline and light fuel oils from naphthenic stock, refiners catalytically split large, high-boiling compounds into smaller molecules and reform them chemically into products with the appropriate volatility, viscosity, and lubricating properties. Mixtures of fractions from different crudes are frequently prepared in order to create the proper physical and chemical properties. The residuals left after distillation and cracking are proportioned into asphalts, coke, some waxes, and bunker oils. These products are likely to contain many large polymers of aromatics, trace metals, and other inorganic.

The No. 2 Fuel oil supplied by the API for many of the above tests, for example, was a mixture of virgin stock (straight run distillation fraction) and cracked stock.

As supplies of paraffinic crudes have been depleted (such as from Pennsylvania), more naphthenic crudes are being used for fuels, and the proportions of aromatics has increased in the refined products. The toxicity of these cracked products is higher than that of simple distilled products.

SUMMARY OF STUDIES ON ISOLATED FRACTIONS OR SPECIFIC COMPONENTS OF OILS:

Results of all component studies indicate that straight or branched alkane hydrocarbons are relatively non-toxic to embryos. Embryo mortality only occurred when the dose of oil was sufficient to coat the egg and prevent oxygen and carbon dioxide gas exchange so that the embryo suffocated.

Large, poly-cyclic aromatic hydrocarbons, however, are extremely toxic. This class of compounds is often referred to as poly-aromatic hydrocarbons (PAH) or poly-nuclear aromatics, and include some of the most carcinogenic compounds known. Benzo(a)pyrene (BaP), chrysene, and dimethylbenz(a)anthracene (DMBA) were shown to be highly toxic, pyrene only slightly less toxic. Chrysene, DMBA, and pyrene are all polymers of four benzene rings (tetracyclics), while Bap is composed of five benzene rings (pentacyclic). There are many methyl-substituted polyaromatics which are very difficult to identify by gas chromatography which may also be in oils at low concentrations.

PAH's are found in highest concentrations in naphthenic and mixed-base crudes, such as South Louisiana, Prudhoe Bay, Venezuela, California, and Kuwait, to list some of the better studied oils. Refined products made from these crudes contain higher levels of aromatics and polyaromatics than products refined from paraffin based crudes.

SUMMARY OF STUDIES OF OILS CONTAINING HEAVY METALS:

Methyl mercury, vanadium and nickel, all of which may be components of crude oils, cause both embryonic mortality and malformations when administered to eggs. Lead is an additional heavy metal which is a component of waste oils which is toxic. Lead and mercury appear to be the most toxic, but study methods and results are sufficiently different to prevent direct comparisons.

Hoffman DJ (1979). Embryotoxic and Teratogenic Effects of Crude Oil on Mallard Embryos on Day One of Development. Bull. Environm. Contain. Toxicol. 22: 632-637.

	DOSE	% SURVIVAL AT 18 DAYS	% ABNORMAL EMBRYOS
CONTROL		99	3
SOUTH LOUISIANA CRUDE (SLCO)	1 UL	57*	2
	5 UL	17*	29*
	10 UL	<1*	64*

(*) denotes this value is significantly different from controls.

Mallard duck eggs were dosed on day 1 of incubation.
Eggs were candled daily and all surviving embryos were broken out on day 18.
Major times of death were on day 4 and days 7-10.
Embryo weights and crown-rump lengths were significantly shorter in oil groups.
Many surviving embryos showed significant malformations including: incomplete ossification of the toes, deformed bills, reduction in the size of the liver lobes, incomplete feather formation, single instances occurred with reduction in the number of ribs, abnormal vertebrae, and spina bifida.

Szaro RC, Albers PH (1978). Petroleum: Effects on Mallard Egg Matchability. J. Wildl. Manage. 42: 404-406.

	DOSE	% SURVIVAL AT: 96HRS	HATCHING
CONTROL		100	88
SLCO	1 UL	70*	62*
	5 UL	12*2*	
	10 UL6*	2*	
	20 UL6*	0*	
	50 UL	0*	0*

(*) denotesthis value is significantly different from controls.
South Louisiana Crude applied to Mallard duck eggs.
Oil applied on day 8 of incubation.
Investigated survival over the first 4 days and survival to hatching.

Significant depression of survival with 1ul, complete hatching failure at 20 ul.
Death of all eggs in 96 hr with 50ul.

Szaro RC (1977). Effects of Petroleum on Birds. Transactions of the 42nd North American Wildlife and National Resources Conference, Wildlife Management Institute, Washington, D.C. pp. 374-381.

Albers PH (1978). The Effects of Petroleum of Different Stages of Incubation in Bird Eggs. Bull. Environm. Contain. Toxicol. 19: 624-630.

	DOSED ON DAY OF INCUBATION	DOSE	% HATCHING
CONTROL			100
SLCO	2	5 UL	0*
	6	5 UL	3*
	10	5 UL	8*
	14	5 UL	78*
	18	5 UL	88*
	22	5 UL	95

(*) denotes this value is significantly different from controls.

South Louisiana Crude applied to Mallard duck eggs at different times during incubation.

100% death of eggs with 5 ul when applied to newly laid eggs.
Progressive tolerance of eggs to oil throughout incubation.
95% survival of embryos when oiled at day 22 (hatching is at day 26).

Hoffman DJ (1978). Embryotoxic Effects of Crude Oil in Mallard Ducks and Chicks. Toxicology and Applied Pharmacology 46: 183-190.

		% SURVIVAL DAY18	% ABNORMAL EMBRYOS
	MALLARD DUCK		
CONTROL		97	4.8
SLCO	1 UL	65*	4.8
	5 UL	9*	66.7*
	CHICKEN		
CONTROL		98	3.5
SLCO	1 UL	62*	40. 3*
	5 UL	20*	79.5*
	10 UL	2*	

(*) denotes this value is significantly different from controls.

South Louisiana Crude dosed eggs on day 2 (chicken) or day 3 (duck) of incubation.
Eggs were broken out on day 18 and examined for survival and abnormalities.
All doses of South Louisiana Crude were toxic and caused abnormalities.
Most common abnormality was incomplete or abnormal ossification of the skull.

Szaro RC, Albers PH (1978). Petroleum: Effects on Mallard Egg Matchability. J. Wildl. Manage. 42: 404-406.

	DOSE	96 HRS.	HATCHING
CONTROL		100	92
KUWAIT CRUDE	1 UL	82*	72*
	5 UL	34*	24*
	10 UL	20*	16*
	20 UL	20*	6*
	50 UL		

(*) denotes this value is significantly different from controls.

Kuwait crude oil applied to Mallard Duck eggs on day 8 of incubation.
Significant mortality with 1 u1.
80% reduction in survival, 84% reduction in hatching with 10 ul.

Lewis SJ, Malecki RA (1983). Reproductive Success of Great Black- Backed and Herring Gulls in Response to Egg Oiling. In "The Effects of Oil on Birds : Physiological Research, Clinical Applications & Rehabilitation." A Multi-discipline Symposium. 1982 Proceedings, Tri-State Bird Rescue & Research, Inc., Wilmington, Delaware, pp. 98-114.

	DOSE APPLIED TO EGGS	% HATCHING SUCCESS GBB GULLS	HERRING GULLS
CONTROL		*83*	*75*
KUWAIT CRUDE	5 UL	85	75
	20 UL	62	57
	50 UL	20*	18*
	100 UL	0*	0*

	DOSE APPLIED TO ADULT GULLS	% HATCHING SUCCESS GBB GULLS	HERRING GULLS
CONTROL		84	73
KUWAIT	1 ML	70	27*
	2 ML	63	43*

(*) denotes this value is significantly different from controls.

Great Black-backed or Herring Gull eggs were dosed at 7-9 days of incubation.
Adult gulls were oiled on the brood patch or dipped in a known quantity of oil.
Kuwait crude was significantly toxic when applied to eggs or adult birds.

Szaro RC, Coon NC, Stout W (1980). Weathered Petroleum: Effects on Mallard Egg Matchability. J. Wildl. Manage. 44(3):709-713.

	DOSE	% SURVIVAL OF EMBRYOS TO HATCHING FRESH CRUDE	WEATHERED 10 DAYS INDOORS	OUTDOORS
CONTROL		74		
PBCO	1 UL	68	76	80
	5 UL	48*	70	60
	10 UL	48*	46*	42*
	20 UL	28*	30*	28*
	50 UL	2*	2*	0*

(*) denotes this value is significantly different from controls.

Prudhoe Bay Crude oil applied to newly laid Mallard duck eggs.
Effect of fresh and weathered oils.
1 ul fresh caused 32% reduction in survival.
Weathering 10 days resulted in 20% reduction in survival.
20 ul of either fresh or 10 day weathered oil resulted in 72% dead embryos.
Longer weathering times resulted in progressive loss of toxicity.

Albers PE (1980). Transfer of Crude Oil from Contaminated Water to Bird Eggs. Envir. Res. 22: 307-314.

	DOSE	HATCHING SUCCESS	CHICK SURVIVAL
CONTROL		96	95
PBCO	5 ML/SQ. METER	80*	98
	100 ML/SQ. METER	47*	98

(*) denotes this value is significantly different from controls.

Mallard ducks were kept in outside pens with water troughs for swimming.
Prudhoe Bay crude oil was added to the troughs at the levels of:
5 ml per square meter water surface (0.005 mm thick, “very light spill”)
100 ml per square meter water surface (0.1 mm thick, “moderate spill”)
Oil was put on troughs on day 5 or 6 of incubation.
Oil was left on water for 48 hr.
Oil was transferred *to* birds, subsequently transferred to eggs, and was toxic.

Albers PH, Gay ML (1982). Effects of a Chemical Dispersant and Crude Oil on Breeding Ducks. Bull. Environm. Contain. Toxicol. 29: 404-411.

	DOSE	% HATCHED
CONTROL		91
PBCO	100 ML/SQ. METER	50*
COREXIT 9527 (1:9)	10 ML/SQ. METER	80
OIL+COREXIT	100+10 ML/SQ. METER	60*

(*) denotes this value is significantly different from controls.

Mallard ducks were kept in outside pens with water troughs for swimming.
Prudhoe Bay crude and Corexit 9527 were sprayed on the water troughs.
Oil, dispersant, or oil+dispersant were added to the troughs at the levels of:
100 ml oil per square meter water surface (0.1 mm thick, “moderate spill”)
10 ml corexit per square meter water surface.
100 ml oil plus 10 ml corexit per square meter water surface.
Oil or dispersant was put on troughs on day 5 or 6 of incubation.
Products were *left* on water for 48 hr.
Oil was transferred to birds, subsequently transferred to eggs, and was toxic.
Dispersant alone was not significantly different from controls.
Oil plus dispersant was toxic to embryos.

Macko SA, King SM (1980). Weathered Oil: Effect on Matchability of Heron and Gull Eggs. Bull. Environm. Contain. Toxicol. 25: 316-320.

		SURVIVAL TO 12DAYS
	LOUISIANA HERON	
	DOSE	
CONTROL		100%
LIBYAN CRUDE (FRESH)	10 UL	27/29 (93%)
WEATHERED 4 WKS	10 UL	19/24 (79%)*
	LAUGHING GULL	
CONTROL		**100%**
LIBYAN CRUDE (FRESH)	10 UL	***32/35 (91%)***
WEATHERED 4 WKS	10 UL	***16/17 (94%)***
WEATHERED 8 WKS	10 UL	***29/31 (94%)***

(*) denotes this value is significantly different from controls.

Libyan crude oil applied *to* Louisiana Heron or Laughing Gull eggs.
Oil was fresh or weathered 4 to 8 weeks.
Fresh and weathered products reduced survival by only 6-9% with gull eggs.
One test with weathered crude on heron eggs resulted in 21% reduction in survival with 10 ul.

STUDIES ON REFINED PRODUCTS:

Szaro RC, Albers PH (1978). Petroleum: Effects on Mallard Egg Matchability. J. Wildl. Manage. 42: 404-406.

		% SURVIVAL AT:	
	DOSE	96 HRS.	HATCHING
CONTROL		100	88
NO.2 FUEL OIL	1 UL	80*	64*
	5 UL	40*	18*
	10 UL	26*	10*
	20 UL	2*	0*

(*) denotes this value is significantly different from controls.

Mallard duck eggs dosed with fuel oil on day 8 of incubation.
Number surviving 4 days and percent hatching.
Significant reduction in survival with 1 ul No. 2 fuel oil.
No eggs hatching with 20 ul oil.

Szaro RC (1977). Effects of Petroleum on Birds. Transactions of the 42nd North American Wildlife and National Resources Conference, Wildlife Management Institute, Washington, D.C. pp. 374-381.

Albers PH (1978). The Effects of Petroleum of Different Stages of Incubation in Bird Eggs. Bull. Environm. Contain. Toxicol. 19: 624-630.

	DOSED ON DAY OF INCUBATION	DOSE	% SURVIVAL TO HATCHING
CONTROL			80
NO. 2 FUEL OIL	2	5 UL	13*
	6	5 UL	33*
	10	5 UL	68*
	14	5 UL	83
	18	5 UL	80
	22	5 UL	93

(*) denotes this value is significantly different from controls.

Mallard duck eggs dosed on various days of incubation.
All eggs dosed with 5 ul No. 2 fuel oil.

5 ul No. 2 fuel oil reduced survival of eggs at two days of incubation to 13%.
Embryos became less sensitive to oil toxicity throughout incubation.

Szaro RC, Albers PH (1977). Effects of External Applications of No.2 Fuel Oil on Common Eider Eggs. In "Fate and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organisms" Edited by Douglas A. Wolfe, Pergamon Press, Inc., N.Y. Chap. 16, pp.164-167.

	DOSE	% HATCHING
CONTROL		96
PROPYLENE GLYCOL	20 UL	96
NO. 2 FUEL OIL	5 UL	92
NO. 2 FUEL OIL	20 UL	69*

(*) denotes this value is significantly different from controls.

Common Eider Duck eggs dosed with No. 2 fuel oil or propylene glycol.
Age of embryos was variable, eggs were collected from the wild.
Proplyene glycol had no effect.
No. 2 fuel oil killed many embryos early in incubation.

White DH, King KA, Coon NC (1979). Effects of No. 2 Fuel Oil on Matchability of Marine and Estuarine Bird Eggs. Bull. Environm. Contain. Toxicol. 21: 7-10.

	DOSE	% SURVIVAL 5DAYS	HATCHING
	LOUISIANA HERON		
CONTROL		95	17
NO.2 FUEL OIL	20 UL	37*	2
	SANDWICH TERN		
CONTROL		100	27
NO.2 FUEL OIL	20 UL	43*	0
	LAUGHING GULL		
CONTROL		98	51
NO.2 FUEL OIL	5 UL		24
	20 UL	17*	4

(*) denotes this value is significantly different from controls.

Eggs were collected in the wild.
Two experiments were done:
1) Marked eggs were dosed with fuel oil in natural nests. Eggs were collected and examined at 5 days after dosing.
2) Eggs were collected and artificially incubated after dosing. Survival to hatching was recorded.

Artificial incubation resulted in many dead controls, but fuel oil was significantly harmful at 5 and 20 ul.

Albers PE (1977). Effects of External Application of Fuel Oil on Matchability of Mallard Eggs. In: Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Ecosystems. Douglas A. Wolfe, ed. Pergamon Press, New York . pp.158-163.

	DOSE	% HATCHING SUCCESS	% OF EGG COVERED
CONTROL		88	
PROPYLENE GLYCOL	50 UL	80	13
NO. 2 FUEL OIL	5 UL	45*	5
	10 UL	12*	12
	20 UL	2*	20
	50 UL	0*	32

(*) denotes this value is significantly different from controls.

Mallard Duck eggs were dosed with oil on day 8 of incubation.
Hatching success and extent of the egg surface covered by oil were recorded.
No. 2 Fuel Oil reduces hatching success

Szaro RC, Coon NC, Stout W (1980). Weathered Petroleum: Effects on Mallard Egg Matchability. J. Wildl. Manage. 44(3):709-713.

		% SURVIVAL OF EMBRYOS TO HATCHING		
	DOSE	FRESH CRUDE	WEATHERED 10 DAYS INDOORS	OUTDOORS
CONTROL		74	74	74
NO. 2 FUEL OIL	1 UL	64	74	78
	5 UL	42*	36*	46*
	10 UL	12*	10*	42*
	20 UL	0*	0*	24*
	50 UL	0*	0*	I)*

(*) denotes this value is significantly different from controls.

Mallard eggs were dosed on day 8 of incubation.
Hatching success was recorded.
Weathering for 2-3 weeks resulted in lowered toxicity.
No. 2 fuel oil is about 4-5 times as toxic as Prudhoe Bay crude.

Albers PH, Szaro RC (1978). Effects of No. 2 Fuel Oil on Common Eider Eggs. Marine Pollution Bull. 9: 138-139.

	DOSE	SURVIVAL AT 7 DAYS POST TRT
CONTROL		98
NO. 2 FUEL OIL	5 UL	94
	20 UL	74

(*) denotes this value is significantly different from controls.

Common Eider Duck eggs were dosed with 5 or 20 ul of oil in wild nests.
Eggs were marked, and all nests were reexamined after 7 days.
All eggs were opened and condition and age of the embryo were recorded.
Embryo age at treatment was 0-17 days.
Mortality was not affected by 5 ul of oil.
20 ul of oil resulted in 20% increase in mortality.
Differences in these results from other experiments are probably due to the fact that ages of embryos varied and younger embryos are more sensitive.

Coon NC, Albers PA, Szaro RC (1979). No. 2 Fuel Oil Decreases Embryonic Survival of Great Black-Backed Gulls. Bull. Environm. Contain. Toxicol. 21:152-156.

	DOSE	SURVIVAL AT 8 DAYS POST TREATMENT
	NATURAL INCUBATION	
CONTROL		89
NO. 2 FUEL OIL	5 UL	81
	20 UL	40*
	ARTIFICIAL INCUBATION	
		SURVIVAL TO HATCHING
CONTROL		72
NO. 2 FUEL OIL	5 UL	44*
	20 UL	20*

(*) denotes this value is significantly different from controls.

Great Black-backed Gull eggs dosed with No. 2 fuel oil.
Two experiments:

1) Eggs dosed in the wild and birds incubated for 8 days after dosing
2) Eggs collected and artificially incubated to hatching after dosing

20 ul but not 5 ul caused increased mortality in the wild.
Both 5 and 20 ul caused increased mortality of artificially incubated eggs.
Embryo mortality was greatest for young embryos.

McGill PA, Richmond ME (1979). Hatching success of great black-backed gull eggs treated with oil. Bird Banding 50: 108- 113.

	DOSE APPLIED TO EGGS	% SURVIVAL AT HATCHING
CONTROLS		78
NO. 2 FUEL OIL	20 UL	24*

(*) denotes this value is significantly different from controls.

Eggs of Great Black-backed Gulls oiled in wild nests.
Eggs were oiled between day O and day 10 of incubation.
Adult birds were allowed to incubate undisturbed after oiling.
Hatching success was observed after 32 days.
Unhatched eggs were broken out to record age at death.
No. 2 fuel oil caused significant mortality early in incubation.

King K, Lefever CA (1979). Effects of Oil Transferred from Incubating Gulls to their Eggs. Marine Pollution Bull. 10: 319-321.

	DOSE APPLIED TO ADULT GULLS	% DEAD EMBRYOS AFTER 5 DAYS
CONTROLS		2
NO. 2 FUEL OIL	2.5 ML	41*

(*) denotes this value is significantly different from controls.

Adult Laughing Gulls were trapped on their nests during incubation.
Gulls were dosed with 2.5 ml No. 2 Fuel Oil or water as controls and set free.
No changes in incubation behavior were noted in oiled birds.
Eggs were collected after 5 days, broken out, and deaths recorded.
No. 2 Fuel Oil is very toxic to eggs even when applied to adults and transferred to eggs.

Lewis SJ, Malecki RA (1983). Reproductive Success of Great Black- Backed and Herring Gulls in Response to Egg Oiling. In "The Effects of Oil on Birds : Physiological Research, Clinical Applications & Rehabilitation." A Multi-discipline Symposium. 1982 Proceedings, Tri-State Bird Rescue & Research, Inc., Wilmington, Delaware, pp. 98-114.

DOSE APPLIED TO EGGS		% HATCHING SUCCESS GBB GULLS	HERRING GULLS
CONTROL		83	75
NO. 2 FUEL OIL	5 UL	78	65*
	10 UL	61*	59*
	20 UL	38*	41*
	50 UL	16*	27*
	100 UL	7*	7*

DOSE APPLIED TO ADULT GULLS		% HATCHING SUCCESS GBB GULLS	HERRING GULLS
CONTROL		84	73
NO. 2 FUEL OIL	1 ML	56*	55
	2 ML	53*	57
	10 ML	29*	7*

(*) denotes this value is significantly different from controls.

Great Black-backed or Herring Gull eggs were dosed at 7-9 days of incubation.
Adult gulls were oiled on the brood patch or dipped in a known quantity of oil.
No. 2 fuel oil was significantly toxic when applied to eggs or adult birds.

Szaro RC (1979). Bunker C Fuel Oil Reduces Mallard Egg Matchability. Bull . Environm. Contain. Toxicol. 22:731-732.

DOSE		SURVIVAL AT: 6 DAYS	HATCHING
CONTROL		100	98
BUNKER C	5 UL	52*	36*
	10 UL	44*	18*
	20 UL	14*	6*
	50 UL	2*	0*

(*) denotes this value is significantly different from controls.

Mallard eggs artificially incubated and dosed on day 8 of incubation.
As little as 5 ul of bunker oil reduced hatching success.
Toxicity of bunker C is only slightly less than:
- No. 2 fuel oil
- South Louisiana Crude
- Kuwait crude

Kopischke ED (1972). The Effect of 2,4-D and Diesel Fuel on Egg Matchability. J. Wildl. Manage. 36: 1353-1356.

	% HATCH
CONTROL (WATER SPRAY)	44
No. 1 DIESEL FUEL SPRAY	0*

(*) denotes this value is significantly different from controls.

Diesel fuel was sprayed on artificially incubated pheasant eggs on day 13.
Amount sprayed was sufficient to wet upper surface of egg and have some drips.
Spray was to simulate herbicide or pesticide applications.
All eggs were killed with No. 1 diesel fuel spray.

Albers PH, Gay ML (1982), Effects of a Chemical Dispersant and Crude Oil on Breeding Ducks. Bull. Environm. Contain. Toxicol. 29: 404-411.

	DOSE	% HATCHING
CONTROL		79
AVIATION KEROSINE	1 UL	79
	5 UL	83
	20 UL	74
WEATHERED AV. KEROSINE	1 UL	74
	5 UL	68
	20 UL	85

No values are significantly different from controls,

Aviation kerosine was applied to mallard duck eggs on day 6 of incubation.
Kerosine was weathered 2-5 days in an accidental spill and collected for study.
Hatching success was recorded.
Neither unweathered nor weathered kerosine affected survival.

Hartung R (1965) Some Effects of Oiling on Reproduction of Ducks. J. Wildl. Manage. 29:872-874.

	DOSE	HATCHING SUCCESS (%)
CONTROLS		89
MEDICINAL MINERAL OIL	2-10 mg	25
	11-15 mg	25
	15.1-36 mg	13

Significance of differences could not be determined from data presented,

Mallard Duck eggs were oiled on day 6 of incubation with medicinal mineral oil.
Eggs were oiled by lightly wiping oil over the entire egg and weighing egg to determine the dose. (10 mg of oil is approximately 10 ul.)
Most eggs were dead 2 days after oiling.
Clogging of pores is the most likely cause of death in this study.

Two adult mallards were oiled with 4-5 ml of medicinal mineral oil in a second experiment and allowed to incubate eggs. All eggs were dead before hatching.

Hoffman DJ, Eastin WC Jr, Gay ML (1982). Embryotoxic and Biochemical Effects of Waste Crankcase Oil on Birds' Eggs . Toxicol. Appl. Pharmacol. 63:230-241.

	DOSE	(%) SURVIVALAT 18 DAYS	% ABNORMAL EMBRYOS
CONTROL		96	2
CLEAN CRANKCASE OIL	15 UL	81*	6
WASTE CRANKCASE OIL	2 UL	83*	10*
	5 UL	51*	22*
	10 UL	16*	56*

BOBWHITE QUAIL

	DOSE	(%) SURVIVAL AT 16 DAYS	% ABNORMAL EMBRYOS
CONTROL		95	5
CLEAN CRANKCASE OIL	3 UL	86*	8
WASTE CRANKCASE OIL	0.5 UL	98	6
	1 UL	64*	16*
	3 UL	12*	33*

(*) denotes this value is significantly different from controls.

Mallard Duck and Bobwhite Quail eggs were dosed with crankcase oil.
Duck eggs were dosed on day 3, Bobwhite eggs were dosed on day 2 of incubation.
Waste crankcase oil was much more toxic than clean oil.
Toxicity was attributed to:
high lead concentration (4600 ppm)
high aromatic content including 386 ppm naphthalene
suspected high content of benzo(a)pyrene
Surviving embryos had many malformations including edema, eye and brain defects, and incomplete ossification.

STUDIES OF ISOLATED FRACTIONS OR SPECIFIC COMPONENTS OF OILS:

Hoffman DJ (1978). Embryotoxic Effects of Crude Oil in Mallard Ducks and Chicks. Toxicology and Applied Pharmacology 46: 183-190.

		SURVIVAL TO 18 DAYS	% ABNORMAL EMBRYOS
MALLARD DUCK			
CONTROL		97	4.8
PARAFFIN MIXTURE	5 UL	94	3.3
CHICKEN			
CONTROL		98	3.5
PARAFFIN MIXTURE	5 UL	96	5.8

No results are significantly different from controls.

Eggs were dosed with a paraffin mixture on day 2 (chicken) or 3 (duck) of incubation.
Eggs were broken out on day 18 and examined for survival and abnormalities.

Paraffin mixture was equal proportions of: pentadecane, hexadecane, heptadecane, octadecane, nonadecane, 2,2,4, 6,6-pentamethylheptane, 2,2,4,4,6,8, 8-heptamethylnonane, 2,6,10, 14-tetramethylpentadecane, and decahydronaphthalene.
Paraffin mixture caused no death or abnormalities.
These paraffins are common straight and branched alkanes of most crude oils.

Szaro RC, Albers PH (1978). Petroleum: Effects on Mallard Egg Matchability. J. Wildl. Manage. 42: 404-406.

	DOSE	% SURVIVAL AT: 4 DAYS	HATCHING
CONTROL		98	92
PROPYLENE GLYCOL	50 UL	100	94
PARAFFIN MIXTURE	50 UL	100	96

No results are significantly different from controls.

Mallard Duck eggs were dosed with a paraffin mixture on day 8 of incubation.
Eggs were candled on day 12 and live embryos were allowed to go to hatching.
Paraffin mixture was equal proportions of: tridecane, pentadecane, hexadecane, heptadecane, octadecane, nonadecane, 2,2,4,6,6-pentamethylheptane, 2,2,4,4, 6,8,8-heptamethylnonane, 2,6,10,14-tetramethylpentadecane, and decahydronaphthalene. (This is the same mixture as the study above with the addition of tridecane).
Paraffin mixture caused no death or abnormalities.
These paraffins are common straight and branched alkanes of most crude oils.

Hatching weights of chicks from these eggs was not different from controls.

Hoffman DJ (1979). Embryotoxic and Teratogenic Effects of Petroleum Hydrocarbons in Mallards. J. of Toxicol. and Environm. Health 5: 835-844.

	DOSE	18 DAYS	SURVIVORS
STUDY 1:			
CONTROL		98	2.5
ALIPHATIC HYDROCARBON MIXTURE	20 UL	96	5.1
AROMATIC HYDROCARBON MIXTURE (52%)	5 UL	86*	4.5
	10 UL	64*	16.6*
	20 UL	31*	18.0*
AROMATIC MIXTURE + 0.5% CHRYSENE	5 UL	41*	37. 5*
STUDY 2:			
CONTROLS		98	4
COMPLETE ALIPHATIC MIXTURE	20 UL	98	2
COMPLETE AROMATIC MIXTURE (52%)	20 UL	42*	18*
MONOCYCLIC AROMATICS	20 UL	98	4
DICYCLIC AROMATICS	20 UL	96	2
TRICYCLIC AROMATIC (PHENANTHRENE)	20 UL	98	2
TETRACYCLIC AROMATIC (PYRENE)	20 UL	86*	6
HETEROCYCLIC AROMATIC	20 UL	100	2
THIOPHENO AROMATICS	20 UL	96	6
STUDY 3:			
CONTROLS		100	3
COMPLETE AROMATIC MIXTURE (52%)	20 UL	43*	18*
MONOCYCLIC + DICYCLICS	20 UL	98	3
MONOCYCLIC + PHENANTHRENE	20 UL	93*	3
MONOCYCLIC + PYRENE	20 UL	88*	6
MONOCYCLIC + THIOPHENES	20 UL	100	5
MONOCYCLIC + HETEROCYCLIC	20 UL	100	3
DICYCLICS + PHENANTHRENE	20 UL	98	5
DICYCLICS + PYRENE	20 UL	90*	6
DICYCLICS + THIOPHENES	20 UL	87*	3
DICYCLICS i- HETEROCYCLIC	20 UL	90*	6
PHENANTHRENE + PYRENE	20 UL	90*	6
PHENANTHRENE + THIOPHENES	20 UL	98	3
PHENANTHRENE + HETEROCYCLIC	20 UL	100	3
PYRENE + THIOPHENES	20 UL	93*	3
PYRENE -I- HETEROCYCLIC	20 UL	98	3
THIOPHENES + HETEROCYCLIC	20 UL	95	5

(*) denotes this value is significantly different from controls.

Mallard duck eggs were dosed on day 3 of incubation.
Eggs were candled daily to record embryo deaths.
Mixtures of aliphatic and aromatic components of similar proportions to SLCO were used in various combinations to assess toxicity.
Aliphatic mixture was equal proportions of: pentadecane, hexadecane, heptadecane, octadecane, nonadecane, 2,2,4, 6,6-pentamethylheptane, 2,2,4,4,6,8, 8-heptamethylnonane, 2,6,10, 14-tetramethylpentadecane, and decahydronaphthalene.
Complete aromatic mixture was 52% aromatics dissolved in the aliphatic mixture and included the following compounds:

Monocyclic: ethylbenzene, pentamethylbenzene, l-phenylhexane, l-phenyltridecane, and tetralin at 4% each.
Dicyclics: dimethylnaphthalene, acenaphthene, acenaphthylene, dibenzofuran, and fluorene at 4% each.
Tricyclic: phenanthrene at 4%.
Tetracyclic: pyrene at 4%
(Chrysene was used at 0.5% in some specific tests).
Thiophenes: benzothiophene and dibenzothiophene at 1.5% each.
Heterocyclic: 2,3,3-trimethylindolenine.

Studies 2 and 3 consisted of tests of individual classes or pairs of classes of aromatics at the concentrations above.

The complete aromatic mixture caused embryonic deaths and abnormalities with a similar temporal pattern as South Louisiana Crude Oil.
No individual class of aromatics alone or in pairs were as toxic as the complete mixture.
Chrysene was much more toxic than other aromatics including pyrene, but the mixtures could not sum to the total toxicity of the crude oil.
Conclusion was that other compounds, probably polycyclic aromatics and methylated derivatives must contribute to the overall toxicity of SLCO.

Hoffman DJ, Gay ML (1981). Embryotoxic Effects of Benzo(a)pyrene, Chrysene, and 7,12-Dimethylbenz (a)anthracene in Petroleum Hydrocarbon Mixtures in Mallard Ducks. J. Toxicol. & Environm. Health 7:775-787.

	DOSE	% SURVIVAL AT 18 DAYS	% ABNORMAL EMBRYOS
CONTROL		100	5
AROMATIC MIXTURE	10 UL	94	4
AROMATIC MIXTURE +	0.02% BaP	93	14*
AROMATIC MIXTURE +	0.1% BaP	36*	31*
AROMATIC MIXTURE +	0.5% BaP	0*	

(BaP = BENZO(A)PYRENE)

CONTROL		98	6
AROMATIC MIXTURE	10 UL	87	11
AROMATIC MIXTURE +	0.05% CHRYSENE	85*	13
AROMATIC MIXTURE -t	0.15% CHRYSENE	71**	28**
AROMATIC MIXTURE +	0.50% CHRYSENE	41**	37**
CONTROL		98	3
AROMATIC MIXTURE	10 UL	92	7
AROMATIC MIXTURE +	0.02% DMBA	76**	15*
AROMATIC MIXTURE -I-	0.10% DMBA	9**	25**
AROMATIC MIXTURE +	0. 50% DMBA	0**	

(DBMA = 7,12 -DIMETHYLBENZ(A) ANTHRACENE)

(*) denotes this value is significantly different from controls.

(**) denotes this value is significantly different from hexane controls.

Mallard eggs were dosed on day 3 of incubation.
Eggs were dosed with an aromatic mixture plus one of three polyaromatics.
Aromatic mixture was 26% aromatics dissolved in an aliphatic mixture and included the following compounds:

Monocyclic: ethylbenzene, pentamethylbenzene, l-phenylhexane, l-phenyltridecane, and tetralin at 2% each.
Dicyclics: dimethylnaphthalene, acenaphthene, acenaphthylene, dibenzofuran, and fluorene at 2% each.
Tricyclic: phenanthrene at 2%.
Tetracyclic: pyrene at 0.5%
Thiophenes: benzothiophene and dibenzothiophene at 0.75% each.
Heterocyclic: 2,3,3-trimethylindolenine at 2%.

The aliphatic mixture was equal proportions of: pentadecane, hexadecane, heptadecane, octadecane, nonadecane, 2,2,4, 6,6-pentamethylheptane, 2,2,4,4,6,8, 8-heptamethylnonane, 2,6,10, 14-tetramethylpentadecane, and decahydronaphthalene.
Addition of benzo(a)pyrene, chrysene, or dimethyl benz(a)anthracene increasedthe embryotoxicity of the mixture of aromatics equal to or in excess of the toxicity of SLCO.
DIMETHYL BENZ(A)ANTHRACENE was more toxic than CHRYSENE which was more toxic than BENZO(A)PYRENE
The polyaromatic hydrocarbons investigated are present in some crude and waste oils at or above the concentrations which were studied in this work.
All of these compounds are extremely toxic.

Ellenton JA (1982). Teratogenic Activity of Aliphatic and Aromatic Fractions of Prudhoe Bay Crude and Fuel Oil No. 2 in the Chicken Embryo. Toxicol. Appl. Pharm. 63: 209-215.

	ADJUSTED DOSE	EMBRYO SURVIVAL TO DAY 15: % DEAD	% ABNORMAL
CONTROL (HEXANE)		12	0
PBCO	10.00 MGEQ	12	0
	5.00 MGEQ	24	0
CONTROL (5 UL ISOPROPANOL)		18	11
PBCO FRACTION 3	3.75 MGEQ	***67****	***39****
	2.50 MGEQ	***39***	***36****
	1.25 MGEQ	***39***	11
CONTROL (5 UL ISOPROPANOL)		19	4
PBCO FRACTION 4	5.00 MGEQ	***33***	***18***
	3.75 MGEQ	***33***	***15***
	2.50 MGEQ	11	***8***
NO.2 FUEL OIL	10.00 MGEQ	4	4
	5.00 MGEQ	24	0
CONTROL (5 UL ISOPROPANOL)		8	5
NO.2 FRACTION 3	2.50 MGEQ	68*	71*
	1.00 MGEQ	29	25
	0.50 MGEQ	36*	17
	0.25 MGEQ	17	10
CONTROL (5 UL ISOPROPANOL)		4	7
NO.2 FRACTION 4	5.00 MGEQ	14	4
	2.50 MGEQ	7	0
	1.00 MGEQ	19	9

(*) denotes this value is significantly different from controls.
Additional results showed significant differences in head length, weight, and crown-rump length when there was not significance in deaths or abnormalities.
Chicken eggs were dosed with oil or fractions of oil on the air cell membrane.
Prudhoe Bay crude and No. 2 fuel oils and fractions of the oils were used.
Fractions were prepared by column chromatography on neutral alumina and sephadex LH-20.

Fraction 3 represented dicyclic, tricyclic and methylated di- and tri-cyclic aromatics (34-40 identified compounds)
Fraction 4 represented tetracyclic, pentacyclic and methylated tetracyclic aromatics (traces of compounds in No.2 fuel oil, 13+ compounds in PBCO)
Doses applied to eggs were fractions reconstituted in hexane or isopropanol to the approximate level of the fraction found in the stock oil.
Fraction 3 of both oils caused the greatest embryo mortality and abnormalities.
Both fractions of both oils were toxic. The compounds in Fraction 4 may be more toxic, but at lower concentrations than in Fraction 3.

STUDIES OF OILS CONTAINING HEAVY METALS:

Hoffman DJ (1979). Embryotoxic Effects of Crude Oil Containing Nickel and Vanadium in Mallards. Bull. Environm. Contain. Toxicol. 23: 203-206.

	DOSE	% SURVIVAL AT 18 DAYS	% ABNORMAL EMBRYOS
CONTROL		97	2
SLCO	1 UL	54*	11*
SLCO+VA(700ppm)	1 UL	47*	36*
SLCO+NI(700ppm)	1 UL	44*	44*

(*) denotes this value is significantly different from controls.

Mallard Duck eggs were dosed on day 3 of incubation.
South Louisiana Crude was used with the addition of 700 ppm vanadium or nickel.
Vanadyl or nickel mesotetraphenylporphine was added to mimic the porphyrin complex which is the naturally occurring complex form.
Addition of V or Ni did not cause greater mortality than crude oil alone.
V and Ni caused greater embryo abnormalities and lower weights of survivors.

Hoffman DJ, Moore JM (1979). Teratogenic Effects of External Egg Applications of Methyl Mercury in the Mallard, *Arias platyrhynchos*. Teratology 20(3):453-462.

	DOSE	% SURVIVAL AT DAY 18	% ABNORMAL SURVIVORS
CONTROL		97	4
ALIPHATIC MIXTURE	10 ul	96	5
ALIPHATICS + METHYL MERCURY AT:	3 Ug	97	19*
	9 Ug	88*	27*
	27 ug	63*	49*
	90 ug	49*	54*

(*) denotes this value is significantly different from controls,

Mallard duck eggs were dosed on day 3 of incubation.
Aliphatic mixture was equal proportions of: pentadecane, hexadecane, heptadecane, octadecane, nonadecane, 2,2,4, 6,6-pentamethylheptane, 2,2,4,4,6,8, 8-heptamethylnonane, 2,6,10, 14-tetramethylpentadecane, and decahydronaphthalene.
Methyl mercury chloride was dissolved in 10% ethyl acetate and added to the aliphatic mixture at 3-90 ug per 10 u1.
Methyl mercury at levels found in some crude oils (30-72 ppm) is very toxic and causes severe malformations of surviving embryos.

Hoffman DJ, Eastin WC Jr, Gay ML (1982). Embryotoxic and Biochemical Effects of Waste Crankcase Oil on Birds' Eggs. Toxicol. & Applied Pharm. 63:230-241.

		MALLARD DUCK	
	DOSE	(%) SURVIVALAT 18 DAYS	% ABNORMAL EMBRYOS
CONTROL		96	2
CLEAN CRANKCASE OIL	15 UL	81*	6
WASTE CRANKCASE OIL	2 UL	83*	10*
	5 UL	51*	22*
	10 UL	16*	56*

BOBWHITE QUAIL

	DOSE	(%) SURVIVAL AT 16 DAYS	% ABNORMAL EMBRYOS
CONTROL		95	5
CLEAN CRANKCASE OIL	3 UL	86*	8
WASTE CRANKCASE OIL	0.5 UL	98	6
	1 UL	64*	16*
	3 UL	12*	33*

(*) denotes this value is significantly different from controls.

Mallard Duck and Bobwhite Quail eggs were dosed with crankcase oil. Duck eggs were dosed on day 3, Bobwhite eggs were dosed on day 2 of incubation.
Waste crankcase oil was much more toxic than clean oil.
Toxicity was attributed to:
- high lead concentration (4600 ppm)
- high aromatic content including 386 ppm naphthalene
- suspected high content of benzo(a)pyrene

Surviving embryos had many malformations including edema, eye and brain defects, and incomplete ossification.

APPENDIX C
Glossary

GLOSSARY

ADRENAL - Endocrine gland adjacent to the kidney which secretes corticosteroid and catecholamine hormones.

ADRENOCORTICOID - Any hormone secreted by the adrenal cortex.

ADRENOCORTICOSTEROID - Any steroid hormone secreted by the cortex of the adrenal gland.

AGGLUTINATION - The phenomenon consisting of the collection into clumps of the cells or particles distributed in a fluid.

ALIPHATIC - Pertaining to linear hydrocarbon chains.

ANEMIA - A reduction below normal in the number of erythrocytes per volume of blood.

ANEMIC - Pertaining to anemia.

AROMATIC - Pertaining *to* hydrocarbon compounds containing benzene groups.

ATRESIA - The process of involution and degeneration of ovarian follicles

AUTOLYSIS - The spontaneous disintegration of tissues or cells by the action of their own autogenous enzymes, such as occurs after death.

BASOPHILIC - Staining readily with basic dyes. Pertaining to granular leukocytes which stain with such dyes.

BASOPHILS - Granular leukocytes which stain with basic dyes.

BIREFRINGENT - Characterized by the power of double refraction.

BLEBS - Cytoplasmic extrusions characteristic of degenerating cells.

BOWEL - Intestine.

BRONCHUS - Intermediate diameter large airways in the lungs. pl. bronchi.

CECUM - A blind diverticular pouch at the junction of the small and large intestines. pl. cecae.

CLOACA - A common passage for fecal, urinary and reproductive discharge.

CORTEX - A distinct outer layer of an organ or other structure.

CORTICAL - Pertaining to cortex.

CORTICOSTERONE - Predominant adrenal cortical steroid hormone of birds. 4-Pregnen-11B, 21-diol-3 ,20-dione.

CORTICOTROPHIC - Exerting a stimulating effect on the cortex of the adrenal.

CORTICOTROPIN - A protein hormone secreted by the anterior lobe of the pituitary with a stimulatory effect on the cortex of the adrenal. Also called ACTH (adrenocorticotropic hormone).

CROP - A diverticulum of the esophagus of birds or, in some species, a region of the gullet having a thickened mucosa.

CULMEN - Measurement of the upper beak from the tip to the junction of the skin at the base of the beak.

DIFFERENTIAL CELL COUNT - Count of white blood cells into proportions of different cell types.

DIMORPHISM - Property of having or existing under two forms.

DISASSOCIATION - Separation of cells normally adherent.

DISTAL - Farther from any point of reference.

EL NINO - The southern oscillation and global atmospheric disturbance responsible for periodic disruptions in sea conditions chiefly of the eastern Pacific ocean.

ENTERITIS - Inflamation of the (small) intestine.
EOSINOPHIL - A granular leukocyte with cytoplasmic granules readily stained with eosin.
EPITHELIUMS - Tissue type covering internal and external surfaces of the body.
ESOPHAGEAL - Pertaining to the esophagus.
ESOPHAGUS - The musculomembranous passage extending from the pharynx to the proventriculus.
ETIOLOGIC - Pertaining to etiology.
ETIOLOGY - The study or theory of the factors that cause disease.
ERYTHROCYTE - Red blood cell.
FIBROSIS - The formation of fibrous tissue.
FOCUS - Chief center of a morbid process. pl. foci.
FOLLICLE - A sac or pouchlike depression. Ovarian follicle - the ovum and its encasing theta cells.
GRANULOCYTES - Leukocytes containing granulaes which stain with one or more specific stains.
GRANULOMA - Tumor-like mass or nodule composed largely of capillaries and fibroblasts, often with inflammatory cells present.
HEINZ BODY - Inclusion body in erythrocyte arising from oxidation and precipitation of hemoglobin.
HEMATOCRIT - The volume percentage of erythrocytes in whole blood.
HEMOGLOBIN - The protein respiratory pigment of erythrocytes.
HEMOLYTIC - Pertaining to hemolysis, the liberation of hemoglobin from erythrocytes.
HEMORRHAGE - Bleeding
HEMOSIDEROSIS - Focal or general increase in iron stores without associated tissue damage.
HEPATIC - Pertaining to the liver.
HEPATITIS - Inflammation of the liver.
HEPATOCELLULAR - Pertaining to liver cells.
HEPATOCYTE - A parenchymal liver cell.
HETEROPHILIC - Staining with a type of stain other than the usual one.
HETEROPHIL A granular leukocyte characterized by granules of variable size and staining properties.
HYPERPLASIA - The abnormal increase in the number of cells in normal arrangement in a tissue,
HYPERTROPHY - The enlargement or overgrowth of an organ or part due to an increase in the size of its constituent cells.
HYPOXIA - Low oxygen content or tension.
INDIVIDUALIZATION - The separation of cells normally adherent.
INFILTRATION - The diffusion or accumulation of substances or cells into a tissue not normal to it.
INTRAATRIAL - Within an atrium.
INTRANUCLEAR - Within the nucleus of a cell.
KUPFFER CELL - Large star-shaped phagocytic reticuloendothelial cells which line the walls of the sinusoids of the liver.
LAMINA PROPRIA - The connective tissue coat of a mucous membrane just deep to the epitheliums and basement membrane.
LEUKOCYTE - white blood cell
LUMINAL - Pertaining to the cavity of a tubular structure.
LYMPHOCYTE - A small mononuclear leukocyte.
LYMPHOCYTOPENIA - Reduction in the number of lymphocytes in the blood.

LYMPHOID - Resembling tissue of the lymphatic system.
MICROPHAGE - Large phagocytic reticuloendothelial cell in walls of blood vessels or within inflamed tissues.
MARINATED - Existing at the margin of the structure.
MEDULLA - The inmost portion of an organ or structure.
MEDULLARY - Pertaining *to the* medulla.
MINERALIZED - Infiltrated with inorganic substance.
MMs - Minerals Management Service.
MONOCYTE - Large phagocytic mononuclear leukocyte.
MUCOSA - A mucous membrane.
MUCOSAL - Pertaining to a mucosa.
NAPHTHENIC - Petroleum type characterized by high content of aromatic hydrocarbons.
NECROPSY - Examination of a body after death.
NECROSIS - Death of tissue, usually in a small localized area.
NECROTIC - Pertaining to necrosis.
NEMATODES - A class of cylindrical helminths (round worms) of the phylum Aschelminthes.
OOCYSTS - The encapsulated fertilized macrogamete of any sporozoon.
OTOSCOPE - An instrument for inspection of the ear.
OVARIAN - Pertaining to the ovary, the female gonad.
OXIDASE - Enzyme which catalyses the simple dehydrogenation of a substrate.
PANCREATITIS - Inflammation of the pancreas.
PNEUMOCONIOSIS - A condition characterized by permanent deposition of substantial amounts of particulate matter in the lungs.
POLYNUCLEAR AROMATIC - Aromatic hydrocarbon class of compounds containing more than three phenyl rings.
PRBO - Point Reyes Bird Observatory
PRE-LAYING EXODUS - That portion of late courtship of Procellariiform birds in which both members of the pair leave the breeding colony for a period of ten to thirty days.
PROLACTIN - Protein hormone secreted by the anterior pituitary with effects on the gonad and behavior.
PROVENTRICULUS - The gastric stomach of a bird.
PYKNOTIC - Pertaining to degeneration of a cell characterized by nuclear shrinkage and chromatin condensation.
RBc - Red blood cell, erythrocyte.
RENAL - Pertaining to the kidney.
RETICULOCYTE - Immature erythrocyte characterized by a basophilic staining reticulum within the cytoplasm.
SAPONIFICATION - The process of conversion of a fat into a soap. Hydrolysis of a glyceride ester into an alcohol and an alkali salt of the ester acid.
SEFI - Southeast Farallon Island
SEM - Scanning electron microscope or microscopy.
SEPTA - pl. of septum, a dividing wall or partition.
STRESSOR - An adverse external influence resulting in a physiological response.
SUBMUCOSA - The layer of areolar tissue situated beneath the mucous membrane.
TARSUS - The portion of the leg between the ankle joint and the foot. Usually elongated in birds.
TERTIARY - Third in order.
TOPHI - pl. of tophus, a chalky deposit of urates found in tissues.

TRAUMA - A wound or injury.
ULCER - A local defect or excavation of the surface of an organ or tissue.
VACUOLATED - Characterized by cells with fluid-filled cavities or spaces in the cytoplasm.
VENTRICULUS - The gizzard of a bird.
VILLUS - A small vascular process or protrusion, especially from the free surface of a membrane.
UCD - University of California, Davis
WBC - White blood cell, leukocyte.